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The Evaluation of Various Bases for Sulfonamide Ointments*

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A method for the estimation of the amount of sulfonamides released from various ointments upon contact with saline or serum for varying lengths of time is described.

SINCE the advent of sulfonamide therapy there has been an increasing interest in the direct application of these compounds to infected or potentially infected areas. The local use of sulfonamides in any type of accessible wound or infection, or as a prophylactic in surgery, is so well established that a review of the literature is unnecessary. The use of sulfonamides in ointment bases has likewise become common practice since in this form they are not only easily applied and held in place but, as Strakosch and Olsen (1) have pointed out, the advantage of such topical application is twofold as compared with oral administration. First, they state, it can be assumed that the local tissue concentration of the drug can be reached with smaller amounts of the drug when thus applied, and second, the possibility of untoward toxic reactions is minimized. Furthermore, the external use of sulfonamide powders has the disadvantage of caking or forming crusts in or about the wound. For these reasons, and because of convenience of use, a variety of sulfonamide pastes and ointments have been used or suggested.

Some sulfonamide ointments have been suspensions of the drug in bases such as petrolatum and lanolin, or in mixtures of petrolatum, lanolin, oils, fats, and waxes. Others have been suspensions in emulsified bases containing, in addition to these constituents, varying quantities of water. Preliminary work has indicated that the emulsion type bases seem to be superior to the hydrocarbon bases and that, of the emulsified bases, the oil-in-water type is more effective than the water-in-oil type. The disadvantage of the hydrocarbon bases and, to some extent, of the water-in-oil emulsions seems to be due to the fact that the oils and the grease form a barrier, allowing little or no

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contact between the drug and the tissues. Although the work of some investigators (2–7) indicates that the oil-in-water emulsion bases are superior, other workers (8, 9) claim better results with the water-in-oil emulsions.

In a recent editorial (10) it has been pointed out that many apparent therapeutic failures of sulfonamides are due to improper topical application of the drug with the resulting low local concentrations of the drug in the tissues of the area applied. Although the amount of sulfonamide applied may be sufficient, the form and manner in which it is applied may be such that absorption is insufficient and therefore bactericidal concentrations of the drug in the infected area are not obtained.

On examination it would seem that the hydrophilic oil-in-water bases offer several physical advantages over the water-in-oil bases. In the former, much of the sulfonamide is dissolved in the aqueous or continuous phase of the emulsion and this presents to the tissues a readily available source of the drug. In the latter type, the water is the dispersed phase, the aqueous part of the emulsion existing as minute droplets or globules each surrounded by the continuous phase of oil. This oily material forms a barrier between the drug, either in solution or as powder particles, and the tissues. Furthermore, the hydrophilic bases, because of their low surface tension, more readily form a continuous surface on the tissues and because of their water and serum miscibility, aid the dissemination of the drug in the wound. It has been pointed out (5, 11) that because of their serum miscibility, these ointments do not interfere with drainage of the wound, nor do they interfere with the normal function of the skin nearly as much as do the greasy ointments which inevitably introduce interfacial tension at the apposition of the mutually immiscible oil and exudate.

It has been illustrated by Gershenfeld and Miller (12), Foley and Lee (13), and Gibson, *et al.* (14), among others, that antiseptics and germicides are generally more active in oil-in-water bases than in water-in-oil bases. Fantus (15) states that greasy ointments are contraindicated in acute inflammatory conditions of the skin because of their "heating quality." He shows that cream ointments or emulsions do not have this objection and further states that the success or failure of the treatment depends upon the proper choice of the base.

In order to evaluate further various sulfonamide ointment bases we have attempted: (*a*) to determine the general availability of the sulfonamide and the rapidity of its release from the different bases when a static surface is exposed to a fluid such as saline or serum under conditions simulating those existing in the wound; (*b*) to correlate the results, if possible, with the bacteriological data obtained in testing these ointments against *Staphylococcus aureus* using the F. D. A. agar cup plate method.

EXPERIMENTAL

In the investigation of the various ointment bases we have attempted to choose representative ointments of the various types, and in the case of the emulsion bases, with varying quantities of water. We have divided them into three classes: the water-free hydrocarbon bases, the water-in-oil emulsions, and the oil-in-water emulsions. Some of these formulas are taken directly from the literature where in some cases they have been recommended as vehicles for sulfonamides, others are official bases such as the U. S. P. Ointment and Hydrous Lanolin, and still others are either extemporaneous preparations or formulas taken from the literature and modified.

The following various formulas are designated by numbers which will be used throughout the paper to identify the bases instead of the entire formulas which are space consuming.

Hydrocarbon Base—Water Free

No. 1.	Lanolin, anhydrous	5%
	White Wax	5%
	White Petrolatum	90%

This is the U. S. P. XII White Ointment (16) generally used as a base in official ointments.

Water-in-Oil Emulsion Bases

No. 2. Hydrous Lanolin

This also is an official preparation (17) containing 30% water emulsified in lanolin.

No. 3.	Arlacel C[1]	5.00%
	Lanolin, anhydrous	5.00%
	Petrolatum, white	32.50%
	Mineral Oil, heavy	7.50%
	Glycerin	5.00%
	Water	45.00%

[1] Sorbitan Monooleate, Atlas Powder Company, Wilmington, Del.

This also is a water-in-oil emulsion containing more water, however, than No. 2.

No. 4.	Absorption Base	22%
	Mineral Oil	6%
	Lanolin, anhydrous	3%
	Petrolatum, white	5%
	Glycerin	5%
	Water	59%

This is an extemporaneous absorption base cream containing more water than either of the previous two emulsions.

Oil-in-Water Emulsion Bases

No. 5.	Cetyl Alcohol	15%
	Propylene Glycol	10%
	White Wax	1%
	Sodium Lauryl Sulfate	2%
	Water	72%

This hydrophilic, washable ointment base has been recommended (18, 19) as a base for sulfonamides.

No. 6.	Cetyl Alcohol	6%
	Lanolin	6%
	Sodium Lauryl Sulfate	2%
	Petrolatum, white	14%
	Mineral Oil, heavy	22%
	Water	50%

This formula contains the same emulsifying agent as No. 5 but contains considerably more oil.

No. 7.	Stearic Acid	25%
	Triethanolamine	2%
	Carbitol	5%
	Lanolin	2%
	Water	66%

This base is a triethanolamine stearate emulsion of the vanishing cream type.

No. 8.	Stearic Acid	15%
	Lanolin	4%
	Mineral Oil, heavy	25%
	Triethanolamine	2%
	Glycerin	5%
	Water	50%

This base, like No. 7, is also a triethanolamine stearate emulsion but containing more oil.

No. 9.	Glycerylmonostearate	18%
	Carbowax 1500[2]	2%
	Glycerin	5%
	Water	75%

This is a simple glycerylmonostearate cream with a high water content.

No. 10.	Glycerylmonostearate	12%
	Lanolin	10%
	Cetyl Alcohol	2%
	Mineral Oil, heavy	25%
	Carbowax 1500[2]	2%
	Glycerin	5%
	Water	44%

This has the same emulsifying agents as No. 9 but contains more oil.

[1] Carbide & Chemicals Corp., New York, N. Y.

Sulfonamide ointments were prepared in 5% and 10% strengths by warming the base to 70° on a water bath, adding the sulfonamide, then removing from the water bath and stirring continuously until cooled to room temperature.

In attempting to estimate the rate and amount of sulfonamide released from the ointment, the following procedure was adopted. Four grams of the ointment was applied evenly to the inside surface of a 6- x 1-inch test tube using a glass rod to spread the ointment in a uniform layer with a circular motion. Twenty-five cubic centimeters of saline or serum was then pipetted into the tube (capacity 60 cc.) and the tubes then placed in an incubator at 37.5° for varying lengths of time. Five tubes were thus prepared with each ointment and one taken for analysis after two, four, six, eight, and twenty-four hours' incubation. The contents of the tube taken for analysis were poured carefully into another tube and agitated by inverting three times. An aliquot of this taken for analysis was used directly or after appropriate dilution depending upon the relative concentration of the drug in the liquid. The amount of sulfonamide in the aliquot was determined colorimetrically by the method of Bratton and Marshall (20) using an electrophotometer[3] and a filter with a maximum transmission at 525 mμ.

Although this method is merely an estimation of the amount of sulfonamide absorbed from the ointment, we have obtained, in preliminary work, fairly uniform and reproducible results by carrying out determinations on a number of series of tubes of the same ointment. For instance, 6 tubes of the same sulfonamide ointment were found to release in twenty-four hours 61, 65, 64, 71, 62, and 57 mg. of the drug in 25 cc. of saline. On this basis we have assumed that the method as given above might be used as a criterion of the ability of various ointment bases to liberate sulfonamides incorporated in them.

The following tables and graphs indicate both the rate and amount of sulfonamide released in the various periods of time designated.

TABLE I.—TOTAL SULFATHIAZOLE (IN MILLIGRAMS) RELEASED INTO 25 CC. OF SALINE BY 5% SULFATHIAZOLE OINTMENTS[a]

Base No.	Hours of Exposure of Ointments to Saline 2	4	6	8	24
1	0.2	0.2	0.3	0.3	0.4
2	1.0	1.0	1.2	1.5	1.7
3	0.3	0.4	0.4	1.4	1.9
4	0.0	0.2	0.4	0.7	2.0
5	12.0	20.0	27.5	31.0	40.0
6	4.0	14.5	26.0	31.0	34.0
7	11.5	17.5	20.0	24.0	35.0
8	13.0	17.0	23.0	25.0	27.5
9	11.0	15.0	20.0	25.0	27.5
10	8.0	11.0	16.0	19.0	22.5

[a] See Fig. 1.

[3] Fisher Electrophotometer, Fisher Scientific Co., Pittsburgh, Pa.

TABLE II.—TOTAL SULFATHIAZOLE (IN MILLIGRAMS) RELEASED INTO 25 CC. OF SALINE BY 10% SULFATHIAZOLE OINTMENTS[a]

Base No.	Hours of Exposure of Ointments to Saline 2	4	6	8	24
1	0.2	0.2	0.3	0.5	0.5
2	0.9	1.2	1.6	1.8	1.8
3	0.7	0.8	1.2	1.4	3.2
4	0.1	0.4	0.8	1.0	3.1
5	19.0	35.0	41.0	43.2	52.0
6	6.0	11.5	32.5	48.5	50.5
7	16.0	31.0	35.0	37.0	43.7
8	13.5	33.0	37.0	36.0	50.5
9	12.0	16.0	23.5	32.0	42.5
10	8.0	12.5	19.0	24.0	37.0

[a] See Fig. 2.

TABLE III.—TOTAL SULFANILAMIDE (IN MILLIGRAMS) RELEASED INTO 25 CC. OF SALINE BY SULFANILAMIDE OINTMENTS[a]

Base No.	Hours of Exposure of Ointments to Saline 2	4	6	8	24
	5% Sulfanilamide Ointments				
1	0.3	0.3	0.4	0.4	0.4
3	1.2	4.3	7.0	7.0	18.5
5	17.0	35.0	37.5	50.2	60.0
7	18.5	40.0	45.0	77.5	82.5
8	25.0	40.0	95.0	95.0	95.0
9	6.25	10.0	21.0	25.0	42.0
	10% Sulfanilamide Ointments				
1	0.5	0.5	0.6	0.7	0.7
3	2.0	7.7	12.0	17.0	40.0
5	23.0	55.0	75.0	90.5	107.5
7	40.0	60.0	90.0	150.0	195.0
8	80.0	125.0	160.0	160.0	185.0
9	10.0	20.0	31.0	45.0	60.0

[a] See Figs. 3 and 4.

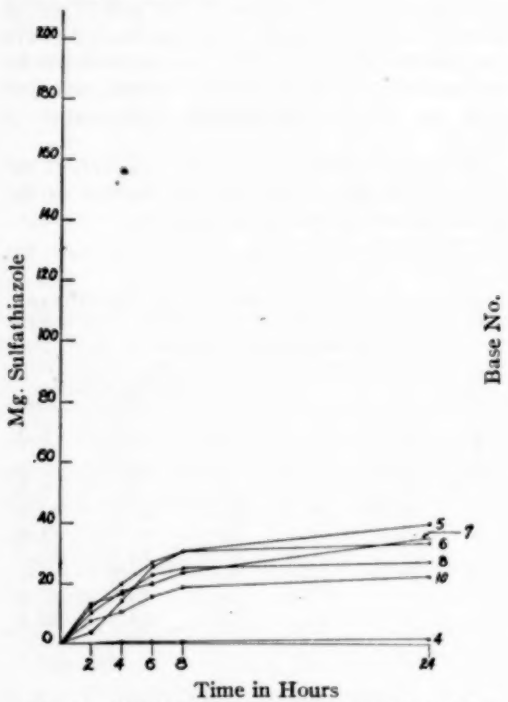

Fig. 1.—Total Sulfathiazole Released into 25 cc. of Saline by 5% Sulfathiazole Ointments.

TABLE IV.—TOTAL SULFONAMIDE (IN MILLIGRAMS) RELEASED INTO 25 CC. SERUM BY SULFONAMIDE OINTMENTS[a]

Base No.	Hours of Exposure of Ointments to Serum 2	4	6	8	24
	10% Sulfathiazole Ointments				
1	0.2	0.2	0.4	0.7	0.7
3	0.5	1.1	2.0	2.6	2.6
5	17.0	39.0	51.0	77.0	92.0
8	9.5	26.5	33.7	34.0	58.7
9	19.0	50.0	55.0	75.0	97.0
	10% Sulfanilamide Ointments				
1	0.2	0.3	0.4	0.6	0.9
3	1.0	4.0	9.0	15.0	19.0
5	62.5	110.0	142.0	187.0	205.0
8	58.0	93.0	137.0	156.0	190.0
9	75.0	120.0	142.0	162.0	192.5

[a] See Figs. 5 and 6.

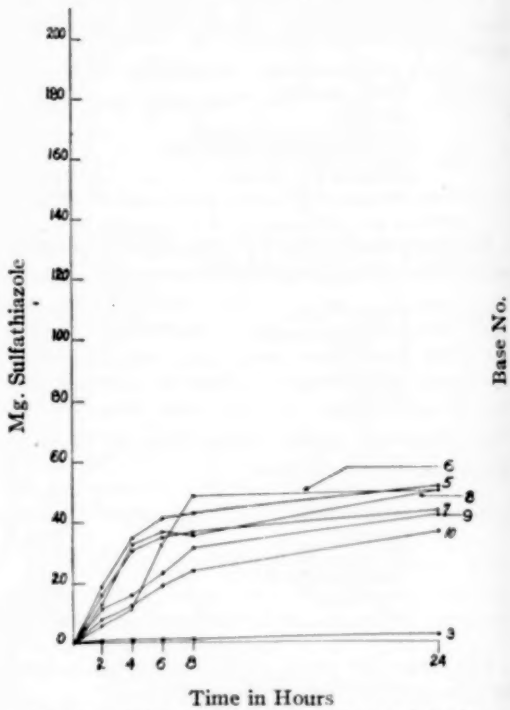

Fig. 2.—Total Sulfathiazole Released into 25 cc. of Saline by 10% Sulfathiazole Ointments.

All ointments were tested bacteriologically against *Staphylococcus aureus* using the F. D. A agar cup plate method (21). Tubes containing 20 cc. of sterile nutrient agar were inoculated with 0.1 cc. of a twenty-four hour culture of *Staphylococcus aureus* at 42° and immediately poured into sterile Petri dishes. After the agar had solidified, 1.5 cm. cups were cut in the center of the plates and 2.0 Gm. of the various ointments introduced. A drop of sterile broth was dropped into the cup to assure uniform contact of the ointment with the agar media. The Petri dishes prepared in this manner were incubated at 37.5° for twenty-four hours. At the end of this period they were removed from the

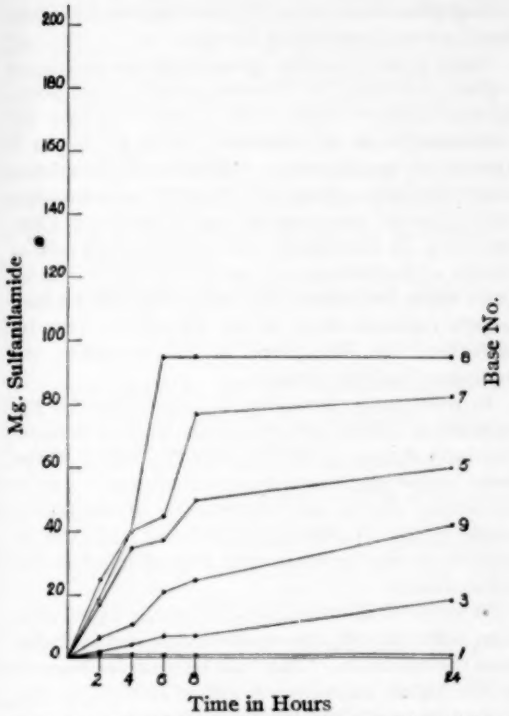

Fig. 3.—Total Sulfanilamide Released into 25 cc. of Saline by 5% Sulfanilamide Ointments.

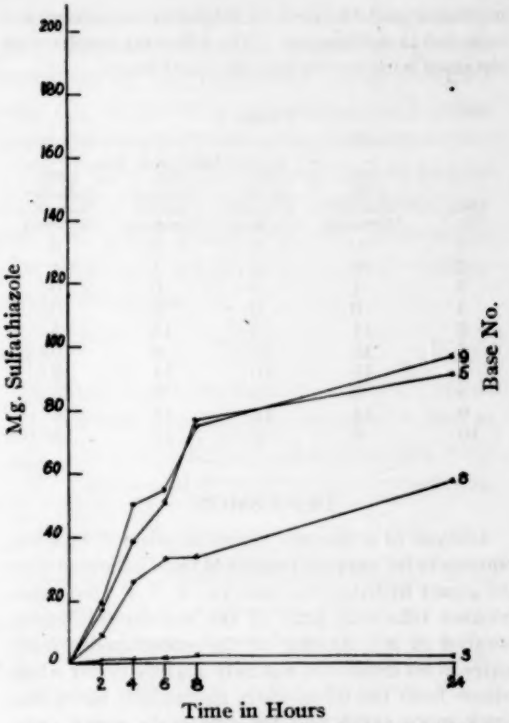

Fig. 5.—Total Sulfathiazole Released into 25 cc. of Serum by 10% Sulfathiazole Ointments.

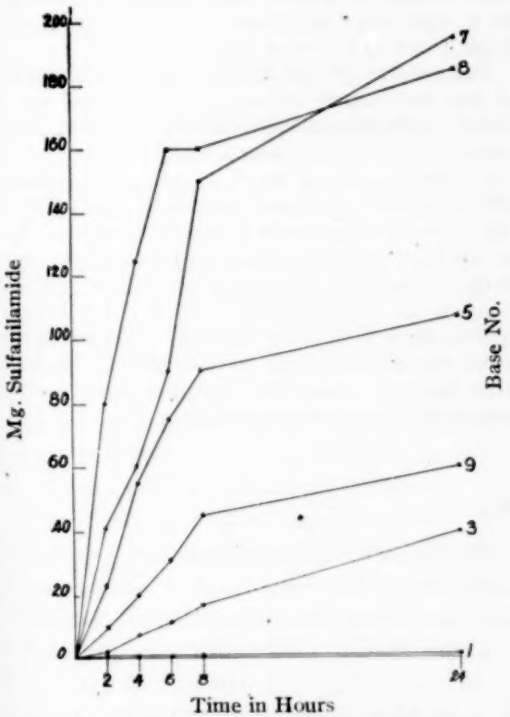

Fig. 4.—Total Sulfanilamide Released into 25 cc. of Saline by 10% Sulfanilamide Ointments.

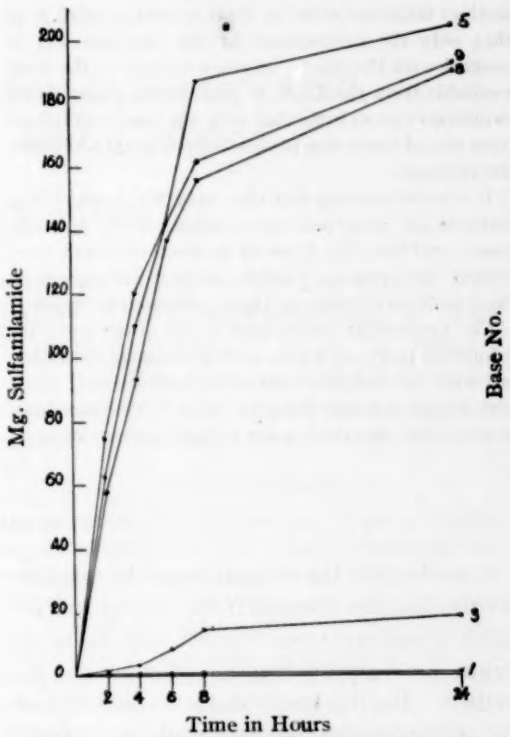

Fig. 6.—Total Sulfanilamide Released into 25 cc. of Serum by 10% Sulfanilamide Ointments.

incubator and the zone of inhibition measured and recorded in millimeters. The following results were obtained with the various ointment bases.

TABLE V

Base No.	Zone of Inhibition, Mm. 5% Sulfathiazole Ointment	10% Sulfathiazole Ointment	5% Sulfanilamide Ointment	10% Sulfanilamide Ointment
1	0	0	0	0
2	3	3	1	1
3	1	1	0	0
4	0	0	0	0
5	14	15	15	15
6	10	10	8	10
7	10	10	11	12
8	6	6	5	7
9	14	15	18	18
10	6	6	12	12

DISCUSSION

Analysis of saline and serum in contact with the ointments for varying lengths of time indicated that the greasy hydrocarbon base, i.e., U. S. P. Ointment, released relatively little of the sulfonamide incorporated in it. Release of the sulfonamides from water-in-oil emulsions was only slightly better while release from the oil-in-water hydrophilic bases was much more rapid and far greater in every case. This is undoubtedly due to the low surface tension and water or serum miscibility of these bases. It was observed that these bases diffused into the saline or serum forming a uniform suspension. The hydrocarbon and water-in-oil emulsions formed a distinct interface with the fluid in contact with it so that only the sulfonamide at the interface was in contact with the fluid. In some instances, the drug available from the U. S. P. base or the water-in-oil emulsions was so little that only the results obtained from one of these was plotted on the graphs to show the contrast.

It is an interesting fact that base No. 3, which is a water-in-oil emulsion containing 50% aqueous phase, and base No. 6, an oil-in-water emulsion containing 50% water, exhibit marked differences in their abilities to give up their sulfonamide content.

The hydrophilic bases used in this study were developed in pairs, each pair having the same emulsifier but with one member containing considerably more petrolatum or grease than the other. This was done to determine whether or not a high concentration of the oil phase interfered with the release of the sulfonamides from ointments of this type.

Bases 5 and 6 have as an emulsifier sodium lauryl sulfate with base No. 6 having a rather high petrolatum content. Bases Nos. 7 and 8 contain triethanolamine as an emulsifier, No. 8 having in it lanolin and petrolatum in addition to the triethanolamine stearate. Bases Nos. 9 and 10 are emulsified with glyceryl monostearate and Carbowax 1500, base No. 10 containing a considerable amount of lanolin and petrolatum not present in No. 9. In all cases where lanolin and petrolatum were added they largely replaced water in the formula so that by increasing the oily phase of the emulsion, the aqueous phase was reduced.

In most cases, increasing the oily phase by the addition of lanolin and petrolatum seemed to interfere only slightly, if at all, with the ability of the bases to give up their sulfonamide content. This is apparently due to the concentration of the sulfonamide in the aqueous phase in which case the oil globules do not interfere with the solubility of the sulfonamides.

As indicated in the graphs and tables, considerably more sulfanilamide than sulfathiazole was available from the ointments. This may be expected because of the higher solubility of sulfanilamide. It also seemed generally true that the ointments released more sulfonamide into serum than into saline. This is undoubtedly due to the higher solubility of these compounds in serum as pointed out by Clark, *et al.* (22).

The oil-in-water bases seemed to give up most of their sulfonamide content quite rapidly in the first six to eight hours and liberated only slightly more in the period up to twenty-four hours.

The water-in-oil emulsions gave up only a fraction of their sulfonamide content very slowly and continued to release small increasing amounts up to and beyond the twenty-four hour period.

Ointments tested by the F. D. A. agar cup plate method against *Staphylococcus aureus* gave inhibition zones which generally paralleled the liberation of the drug from the various ointments by the method described. Ointments which liberated a large amount of sulfonamide gave the largest inhibition zones while those liberating small amounts of sulfonamide gave little or no zone of inhibition. Here, again, it seemed that the oil-in-water emulsions were the most satisfactory bases.

SUMMARY

A method for the estimation of the amount of sulfonamides released from various sulfonamide ointments upon contact with saline or serum for varying lengths of time is described. By this method, the availability of the sulfonamides incorporated in various ointment bases is estimated.

The types of bases studied were a hydrocarbon water free base (U. S. P. Ointment), water-in-oil emulsion bases and oil-in-water emulsion bases. Ointments were prepared by incorporating 5 per cent and 10 per cent of sulfanilamide or sulfathiazole in the various bases. Of these ointments the oil-

in-water emulsion type was most efficient, liberating its sulfonamide content more rapidly and more completely than the others. The hydrocarbon base was the poorest in this respect and the water-in-oil emulsions only slightly better. Increasing the oil phase of the oil-in-water emulsion bases did not seem to materially affect the liberation of the sulfonamides.

All ointments were tested against *Staphylococcus aureus* using the F. D. A. agar cup plate method. Largest zones of growth inhibition of the organisms were obtained with the oil-in-water emulsions with other bases showing little or no inhibition.

From these results it seems logical to conclude that because of their low surface tension and water miscibility, the oil-in-water type emulsions are superior bases for sulfonamide ointments.

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The Separation of Acetylsalicylic Acid, Acetophenetidin, and Caffeine*

By KENNETH E. HOLT†

A FREQUENT operation of the pharmaceutical control laboratory is the separation of mixtures containing acetylsalicylic acid, acetophenetidin, and caffeine. Although the present A. O. A. C. method (1) produces satisfactory results the hydrolysis of acetophenetidin is time-consuming and affords considerable chance of error.

The method outlined here eliminates the hydrolysis of acetophenetidin by utilizing the relative insolubility of caffeine in ether to effect the separation of caffeine from acetylsalicylic acid and acetophenetidin, which are freely soluble in ether. The results obtained are comparable to those obtained with the A. O. A. C. method (1, 3) while the time required for the complete analysis by an experienced analyst is reduced to less than three hours.

* Received May 23, 1945, from the Chemical Laboratories, Cole Chemical Company, St. Louis, Mo.
† Acknowledgment is made to Wendell L. Holt and Margaret Miller for their suggestions.

EXPERIMENTAL

Acetophenetidin.—Accurately weigh a sample containing about 0.3 Gm. of acetophenetidin and transfer to a separatory funnel. Add 25 cc. of HCl (1 + 10) and extract with purified ether using one 40-cc. portion and five 20-cc. portions. Combine ether extracts in another separatory funnel and wash with one 10-cc. portion HCl (1 + 10) and one 10-cc. portion of water, washing these in turn through two 25-cc. portions of ether and adding to the original acid solution. (Save the acid solution

for caffeine.) Combine the ether solutions and extract the acetylsalicylic acid with one 25-cc. portion of 5% $NaHCO_3$ and two 10-cc. portions of water, washing each through two 25-cc. portions of ether and combining in another separator. (Save the aqueous solutions for acetylsalicylic acid.) Filter all ether extracts through a cotton pledget into a tared flask. Evaporate ether, dry at 100°, cool, and weigh as acetophenetidin.

Caffeine.—Extract the original acid solution with four 30-cc. portions of chloroform, washing each in turn through 5 cc. 2% NaOH and 5 cc. water. Filter the chloroform extracts through a cotton pledget into a tared flask. Evaporate the chloroform, dry at 100°, cool, and weigh as anhydrous caffeine.

Acetylsalicylic Acid.—Acidify the $NaHCO_3$ solutions with dilute HCl and extract with one 40-cc. and four 15-cc. portions of ether or chloroform. Wash each through 5 cc. of water and filter into a tared flask through a cotton pledget. Evaporate solvent under reduced pressure, dissolve residue in 10 cc. neutral alcohol, and titrate acetylsalicylic acid using A. O. A. C. double titration method (2).

RESULTS

TABLE I.—SEPARATION OF ACETYLSALICYLIC ACID, ACETOPHENETIDIN, AND CAFFEINE MIXTURES

	Acetylsalicylic Acid			Acetophenetidin			Caffeine		
	Gm. Present	Gm. Found	%	Gm. Present	Gm. Found	%	Gm. Present	Gm. Found	%
No. 1	0.5000	0.4944	98.9	0.3000	0.2986	99.5	0.0500	0.0508	101.6
No. 2	0.6000	0.5961	99.4	0.3500	0.3515	100.4	0.0300	0.0305	101.7
No. 3	0.8000	0.7906	98.8	0.4000	0.4016	100.4	0.0200	0.0207	103.5
No. 4	0.5000	0.4944	98.9	0.2000	0.2004	100.2	0.0500	0.0495	99.0
No. 5	0.5000	0.4926	98.5	0.3000	0.3005	100.2	0.0400	0.0420	105.0
No. 6	0.5000	0.4960	99.2	0.3000	0.3019	100.6	0.0400	0.0402	100.5

The acetylsalicylic acid, acetophenetidin, and caffeine used to obtain the results given in Table I were desiccated, U. S. P. grade chemicals. The ingredients were mixed in the approximate proportions found in pharmaceutical preparations of this type. Special care was taken to use ether as nearly free of alcohol and chloroform as possible because of the solubility of caffeine in these reagents.

Using the quantities of solvents outlined in the foregoing procedure, several trials were made in which caffeine was the only ingredient present, varying in quantities from 0.02 to 0.05 Gm. It was found that 0.0013 to 0.0015 Gm. was extracted and retained by the ether, indicating that the amount of caffeine weighed as acetophenetidin is dependent on the quantity of ether used and not on the amount of caffeine in the sample.

Similarly, in trials in which acetophenetidin was the only ingredient, approximately 0.5% of the acetophenetidin was found to remain in the original acid solution and washes. If the sample contains about 0.3 Gm. of acetophenetidin, the amount of acetophenetidin weighed as caffeine will compensate for the caffeine removed by the ether. These small discrepancies, however, are minimized in routine analysis by the technique of the operator.

SUMMARY

A method is presented for separation of mixtures containing acetylsalicylic acid, acetophenetidin, and caffeine. The mixture is dissolved in an acid solution and the acetylsalicylic acid and acetophenetidin separated from the caffeine with ether. The acetylsalicylic acid is then removed from the acetophenetidin with sodium bicarbonate.

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Action of Cholic-, Desoxycholic-, Dehydrocholic-, and Dehydrodesoxycholic Acids on Body Temperature in Rabbits*

By E. GEIGER, G. SCHNAKENBERG, and L. A. GEIGER

Intravenously injected sodium dehydrocholate solutions cause hypoglycemia as long as the body temperature remains unchanged. Larger doses decrease the body temperature and the hypothermia is accompanied by an increase in the blood sugar values. Dehydrocholic and dehydrodesoxycholic acids have a stronger hypothermic effect than cholic or desoxycholic acid.

SINCE the experiments of Neubauer (1) and Pohl (2), dehydrocholic acid has been extensively used in therapy as a relatively nontoxic, but effective, choleretic agent. It seems that, aside from the action on bile production, the only other property of this acid which has interested investigators (3) so far is its effect on blood sugar regulation. The results of these experiments were not uniform, since in some cases hypoglycemia and in others a rise in the blood sugar values was observed after injection of dehydrocholic acid (4).

A close interrelation between blood sugar regulation and changes in body temperature had been established earlier (5). Therefore, we investigated whether or not after injection of some bile acids, the changes in body temperature could be responsible for the variation of the blood sugar values just mentioned.

EXPERIMENTAL

Preparation.—The cholic acid (m. p. 201°, cor.) and the desoxycholic acid (m. p. 171°, cor.) were isolated from ox bile. The dehydrocholic (m. p. 240°, cor.) and the dehydrodesoxycholic (m. p. 188°, cor.) acids were prepared by chromic acid oxidation of the corresponding hydroxy acids. For injection, the sodium salts were dissolved in freshly prepared distilled water (U. S. P. XII) or in physiological saline solution.

The rabbits originated from a special stock, tested and reserved for routine pyrogen tests (U. S. P. XII). Before the assay, they were starved for sixteen to twenty hours.

* Received Nov. 12, 1945, from the Van Camp Laboratories, Terminal Island, Calif.

The blood for the sugar determinations (Folin-Wu) was taken from the marginal ear vein.

Methods.—In the first set of experiments, we slowly injected 10 ml. of saline containing different amounts of sodium dehydrocholate (Table I). Small doses induced a slight fall in the blood sugar values, with no change in body temperature. Larger doses decreased the temperature and simultaneously produced hyperglycemia.

These results seem to indicate that dehydrocholic acid causes hypoglycemia only while the body temperature remains unchanged. On the other hand, after larger doses, a hypothermia is accompanied by an increase in the blood sugar values. These observations might explain why, according to earlier authors (4) the injection of dehydrocholic acid was followed sometimes by hypo-, and sometimes by hyper-glycemia.

Earlier experiments (5) showed that decreasing the body temperature of rabbits and dogs by cooling, by administration of antipyretics or other drugs is followed by a rise in the blood sugar concentration. The experiments in which dehydrocholic acid decreased the body temperature seem to be in best accordance with those earlier findings. Whether or not the mechanism of the hyperglycemia caused by dehydrocholic acid is analogous to that which prevails after the administration of antipyretics could be determined on splanchnicotomized animals.

The extent of the fall in temperature after sodium dehydrocholate injection, though certainly outside of the limits of possible experimental error, was not very striking. Therefore, in the following experiments, the dehydrocholate was dissolved in a pyrogenic 5% glucose solution. In Table I, Experiments 9 and 10 show that 5 ml. of such glucose solution raised the temperature very considerably. However, simultaneous injection of dehydrocholate (Nos. 11 to 13) in 3 other animals had a very pronounced antipyretic action.

As to the mechanism of the antipyretic activity, a peripheral vasodilation can be excluded as a primary cause. The ears of the animals were very cold after the injection of the bile acids, and the blood vessels were so contracted that it was difficult to obtain the blood necessary. We found also that a pre-treatment of the rabbits with relatively high doses of atropine (2 mg. per Kg., intravenously) did not inhibit the hypothermic action of dehydrocholic acid.

It was reported earlier that in jaundice, hypothermia often may be found (6). Since, in those cases, a retention of bile acids occurs, we investi-

gated whether normal constituents of bile, such as cholic and desoxycholic acid, are also able to decrease the body temperature.

Doses of 80, 90, 100, 125, and 170 mg. of cholic acid per Kg. body weight in form of the Na salt in 10 ml. of saline solution were injected intravenously. The effect of higher doses could not be investigated because 180–200 mg. per Kg. proved to be fatal. Gillert (7) found the toxic dose to be 50 mg. per Kg., which is much lower than that established by us. Possibly Gillert's cholic acid sample contained, as often happens, the much more toxic desoxycholic acid as an impurity. This cannot be the case in our experiments, since we purified the sample by recrystallizations until a negative benzaldehyde reaction (8) proved the absence of desoxycholic acid.

TABLE I.—EXPERIMENTS WITH SODIUM DEHYDROCHOLATE

Expt. No.	Wt. of Rabbit, Kg.		Before Injection 1 Hr.	Before Injection ½ Hr.	After Injection 1 Hr.	After Injection 2 Hr.	After Injection 3 Hr.	Injected
1	3.2	Temp., ° C.	39.8	39.6	39.4	39.4	39.4	180 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	113	117	86	81	
2	2.2	Temp., ° C.	39.5	39.6	39.4	39.4	39.6	182 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	106	93	80	91	
3	2.7	Temp., ° C.	39.3	39.6	39.4	39.3	39.5	122 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	98	104	98	85	
4	3.1	Temp., ° C.	39.2	39.2	39.3	39.2	39.1	120 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	103	97	80	82	
5	2.9	Temp., ° C.	39.7	39.8	38.4	37.4	38.9	345 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	108	124	132	134	
6	2.5	Temp., ° C.	39.5	39.6	38.4	37.2	39.5	400 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	103	144	129	103	
7	2.8	Temp., ° C.	39.6	39.4	38.9	38.2	38.1	480 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	106	138	145	126	
8	2.4	Temp., ° C.	39.1	39.3	39.0	37.9	38.2	360 mg./Kg. Sodium Dehydrocholate
		Blood sugar, mg. %	...	99	160	151	116	
9	2.7	Temp., ° C.	39.6	39.5	40.5	41.4	...	5 ml. Pyrogen-glucose solution
10	3.1	Temp., ° C.	39.5	39.3	40.4	40.3	...	5 ml. Pyrogen-glucose solution
11	3.0	Temp., ° C.	39.1	39.2	37.2	39.8	...	5 ml. Pyrogen-glucose solution + 300 mg./Kg. Na-Dehydrocholate
12	1.9	Temp., ° C.	39.5	39.5	37.3	39.3	...	5 ml. Pyrogen-glucose solution + 510 mg./Kg. Na-Dehydrocholate
13	2.8	Temp., ° C.	39.4	39.6	38.4	39.2	...	5 ml. Pyrogen-glucose solution + 380 mg./Kg. Na-Dehydrocholate

TABLE II.—EXPERIMENTS WITH NA-CHOLATE AND DESOXYCHOLATE

Expt. No.	Wt. of Rabbit, Kg.		Before Injection 1 Hr.	Before Injection ½ Hr.	After Injection ½ Hr.	After Injection 1 Hr.	After Injection 2 Hr.	After Injection 3 Hr.	Injected, mg./Kg.
1	2.6	Temp., ° C.	39.6	39.4	39.4	39.1	39.4	...	Sodium Cholate, 80
		Blood sugar, mg. %	...	95	111	...	106	...	
2	3.1	Temp., ° C.	39.4	39.3	39.6	39.4	39.0	...	Sodium Cholate, 80
		Blood sugar, mg. %	...	84	...	115	105	...	
3	2.9	Temp., ° C.	39.5	39.3	39.1	38.2	38.2	39.3	Sodium Cholate, 90
		Blood sugar, mg. %	...	103	...	112	...	108	
4	2.7	Temp., ° C.	39.4	39.5	39.6	39.5	39.4	...	Sodium Cholate, 100
		Blood sugar, mg. %	...	98	...	104	112	...	
5	2.6	Temp., ° C.	39.6	39.2	38.9	37.4	37.4	37.8	Sodium Cholate, 125
		Blood sugar, mg. %	...	106	118	...	128	...	
6	2.8	Temp., ° C.	39.2	39.3	39.0	39.1	39.2	...	Sodium Cholate, 170
		Blood sugar, mg. %	106	...	115	118	110	...	
7	3.0	Temp., ° C.	39.1	38.9	39.2	39.1	39.1	...	Na-Desoxycholate, 7
		Blood sugar, mg. %	...	98	109	101	...	...	
8	2.8	Temp., ° C.	39.3	39.2	39.6	39.2	39.3	...	Na-Desoxycholate, 10
		Blood sugar, mg. %	...	102	104	115	...	...	
9	3.2	Temp., ° C.	39.4	39.1	39.3	39.5	39.2	...	Na-Desoxycholate, 12
10	2.9	Temp., ° C.	38.9	39.2	38.9	39.3	39.1	...	Na-Desoxycholate, 13
11	3.2	Temp., ° C.	39.2	39.4	39.3	39.2	39.4	39.1	Na-Desoxycholate, 13
		Blood sugar, mg. %	...	98	104	110	...	120	

These cholate injections decreased the body temperature of two rabbits only. In the other cases, the temperature remained constant. The blood sugar concentration, apart from an insignificant thermic activity in the rabbit. It would be interesting to determine by the use of partially oxidized compounds (9) which groups in the bile acid molecule are responsible for this effect.

TABLE III.—EXPERIMENTS WITH NA-DEHYDRODESOXYCHOLATE

Expt. No.	Wt. of Rabbit, Kg.		Before Injection 1 Hr.	Before Injection ½ Hr.	After Injection ½ Hr.	After Injection 1 Hr.	After Injection 2 Hr.	After Injection 3 Hr.	Injected Na-Dehydrodesoxycholate mg./Kg.
1	2.8	Temp., ° C.	39.5	39.4	39.5	39.8	39.8	39.8	50
		Blood sugar, mg. %	...	98	105	103	108	...	
2	2.7	Temp., ° C.	39.4	39.6	38.8	38.3	38.2	38.6	75
		Blood sugar, mg. %	...	101	141	125	...	115	
3	3.1	Temp., ° C.	39.2	39.1	38.4	38.2	37.8	38.1	100
		Blood sugar, mg. %	...	108	...	174	...	116	
4	2.6	Temp., ° C.	39.1	39.2	38.3	38.6	38.1	38.2	130
		Blood sugar, mg. %	...	106	...	200	168	144	

hyperglycemia, did not show any remarkable changes (Table II, Experiments 1 to 6). Desoxycholic acid was injected in doses of 7, 10, 12, and 13 mg. per Kg. Doses of 15–20 mg. per Kg. proved to be fatal, which is in good agreement with Gillert's results (Table II, Experiments 7 to 11). After injection of sodium desoxycholate, we were not able to observe any significant change either in body temperature or in the blood sugar values.

These results indicated that the salt of dehydrocholic acid has a stronger effect on the temperature than that of cholate. Therefore, we investigated whether the hypothermic action of dehydrodesoxycholic acid is also stronger than that of desoxycholic acid. This acid was injected in doses of 50, 75, 100, and 130 mg. per Kg.; 150–180 mg. per Kg. proved to be the fatal dose.

The experiments reported in Table III show that 50 mg. per Kg. increased slightly the body temperature without influencing the blood sugar. Higher doses decreased the body temperature and increased the blood sugar considerably.

The present experiments prove that, like dehydrocholic acid, the keto-derivative of desoxycholic acid, viz., dehydrodesoxycholic acid, possesses hypothermic activity in the rabbit. It would be interesting to determine by the use of partially oxidized compounds (9) which groups in the bile acid molecule are responsible for this effect.

SUMMARY

Intravenously injected sodium dehydrocholate in medium and sublethal doses decreases the body temperature. Simultaneously, with this hypothermia, a marked hyperglycemia develops. Dehydrocholic and dehydrodesoxycholic acid have a stronger hypothermic effect than cholic or desoxycholic acid.

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Peroxide Test for Mineral Oils*

By MILTON J. GOLDEN†

A test solution containing ferrous sulfate and ferrous thiocyanate is added to samples of mineral oil, previously heated for varying periods of time to 300° F., and the developing color observed. Interpretation of the test in terms of stability and expected shelf life of mineral oil is described.

It is known that mineral oil, upon prolonged standing or exposure to sunlight, will develop a peculiar kerosene-like odor and taste. This is believed to be due to the formation of peroxides within the oil (1). The development of peroxides is less likely to occur in highly purified oils than in inferior types. It is imperative that pharmaceutical mineral oils to be taken internally should be stable for a prolonged period of time in respect to odor, flavor, and the development of peroxides.

Although several methods have been proposed for determining the stability of a mineral oil, apparently none has been approved as yet. The Pharmacopœia of the United States provides tests for determining such impurities as solid paraffins, sulfur compounds, and carbonizable substances in mineral oil, but does not include any test for the stability or shelf life of mineral oil, due to the fact that this property has probably been difficult to attain or has been disregarded because of technical difficulties in its manufacture. It was therefore deemed advisable to devise a method whereby a prognosis can be made as to the stability of a given oil by a chemical determination of peroxides formed within the oil after heat treatment.

Formerly, oil stability was tested by exposure to direct sunlight or to heat with subsequent detection of the presence of odors. This method was crude and time-consuming, requiring weeks before any appreciable odor or deterioration could be noticed. Samples of heavy Liquid Petrolatum U. S. P., of various brands, were exposed in filma-sealed, flint bottles to sunlight and to ultraviolet light by Green and Schoetzow (2) and observed for the possible presence of odors and flavors.

Shau and Nielsen (3) investigated the stability of several mineral oil samples spectroscopically and found that they gave a maximum ultraviolet absorption band around 2730 Å. and a minimum at 2500 Å. They compared the degree of absorption with the presence of carbonizable impurities and observed that the color depth of the sulfuric acid test for carbonizable substances was in direct proportion with the absorption, inferior oils giving a maximum absorption.

In 1931, Arditti (4) studied the stability of paraffin oil, free of sulfur compounds, by oxidation with air. Air was bubbled through the oil, preheated at various temperatures, and the change in interfacial tension between a layer of 0.02 *N* sodium hydroxide and the oil was measured. This interfacial tension underwent marked changes after exposure of the oil to 123° for three hours or to 150° for one-half hour.

The methods of both Shau and Nielsen and Arditti are not practical as routine examinations, since they require special equipment and skilled workers. Where the previously mentioned tests for determining the stability of mineral oils are based upon certain physical factors, stability can be determined chemically by the oxidation of the ferrous ion to the ferric ion by peroxides formed in heat-treated oils. The developing pink or red color can then be measured colorimetrically.

Several oil companies and individual investigators (1) have used a colorless test solution of ferrous sulfate, ammonium sulfocyanate, and sulfuric acid for the detection of peroxides. The oils were heated either under steam pressure or by direct

* Received Oct. 5, 1945, from the Research Laboratories, McKesson & Robbins, Inc., Bridgeport, Conn.

† The author expresses his appreciation to Dr. Erich Meyer, of L. Sonneborn Sons, Inc., New York, for his assistance and suggestions.

heat. The test solution was then added, the development of a pink or red color indicating the presence of peroxides, which constituted an objection with regard to stability.

EXPERIMENTAL

The above facts and reactions were used in developing the following proposed method, expressing the expected shelf life or stability of the mineral oil in time units. Samples of mineral oil were left in a 300° F. oil bath for various time intervals, from two minutes to twenty-five minutes. Subsequently, the test solution, consisting of ferrous sulfate, ammonium thiocyanate, sulfuric acid, reduced iron filings, and iron wire is added, and the developing color observed. The color will vary from colorless to dark red, depending upon the amount of peroxides formed by the breakdown of the oil.

Apparatus.—One-liter round-bottom flask and reflux condenser; test tubes ¾ inch by 6 inches marked for 10-cc. filling; constant temperature oil bath regulated at 300° F. ± 1° F.

Materials.—Ferrous Sulfate C. P.; Ammonium Thiocyanate, C. P.; Iron by Hydrogen, Merck (reduced iron); Iron Wire No. 30, for standardization; Acetone U. S. P.

Preparation of Test Solution.—In a round-bottom, wide-mouthed flask, 2 Gm. of ferrous sulfate is dissolved in 100 cc. of distilled water containing 2 cc. concentrated sulfuric acid. Two hundred cubic centimeters of acetone and 100 cc. of a 2% aqueous solution of ammonium thiocyanate are then added to the above solution. Iron powder 0.2 Gm. and 6 inches of No. 30 iron wire are finally added to reduce any trace of ferric ion present. The resulting mixture is refluxed on a steam bath until the red color of the solution has disappeared. This reduction takes about two hours, it being necessary to continue refluxing to insure lack of color.

Fresh solution is prepared on the day on which the test is to be carried out. However, it is possible to preserve it in a colorless state by careful bottling with carbon dioxide.

Since it is necessary to avoid any contact with air, a method has been devised to dispense this solution. A syphon is passed into the upper end of the reflux condenser through a two-hole cork stopper. The second hole is fitted with a glass stopcock, which serves as a vent. Carbon dioxide is admitted through the vent to inaugurate the flow of the solution, the pressure forcing the liquid into the syphon, at the end of which is attached a glass stopcock to withdraw the solution.

Procedure.—Five cubic centimeter samples of the oil to be tested are placed in six individual test tubes, which are immersed in a constant temperature oil bath at 300° F. ± 1° F. Individual tubes are removed after two, five, ten, fifteen, twenty, and twenty-five minutes' immersion in the oil bath. The air is replaced with carbon dioxide, and 5 cc. of the test solution is added. The air in the tube is again replaced with carbon dioxide. The tube is tightly stoppered with a new cork stopper and shaken by hand for fifteen seconds. The presence of peroxides in the oil samples is detected by the development of a pink to red coloration of the bottom layer in the test tubes. The first tube exhibiting a pink coloration after shaking with the test solution is regarded as the Peroxide Test value, expressed in minutes.

DISCUSSION

This peroxide test for mineral oils has been used for several years in this Laboratory to insure the highest stability and purity of medicinal mineral oils and has been evaluated so that minutes of heating and detection of the degree of peroxides formed provide a means for establishing the stability of a certain mineral oil for various lengths of time.

If an oil develops peroxides after two minutes' heating, it is considered unstable and of poor shelf-life and medicinal quality. On the other hand, an oil which gives a colorless test with the ferrous thiocyanate solution after being heated twenty minutes or longer is regarded as stable and of very good quality.

Samples of mineral oil of several brands with varying peroxide test values were stored under normal drugstore environmental conditions and tested frequently for stability as indicated by the development of odor, flavor, and of medicinally undesirable peroxides.

Table I summarizes the coordination of the values obtained in our test with the observed shelf life and stability.

TABLE I.—COORDINATION OF PEROXIDE TEST WITH SHELF LIFE AND STABILITY OF MINERAL OILS

Sample of Various Brands	Peroxide Test in Min.	Approximate Stability	Shelf life
A	1–2	1–2 mo.	Poor
B	5	6–8 mo.	Fair
C	10	10 mo.	Good
D	15	1 yr.	Good
E	20 and over	2 yr. and over	Excellent

This suggested peroxide test offers a means of determining a new constant or property for medicinal mineral oils. A comparison of expected stability of different oils is thereby made possible.

In the pharmaceutical use of mineral oil, it is of utmost importance that careful consideration be given to the factor of stability. The values of the peroxide test offer themselves as a yardstick in this respect. A peroxide test of fifteen minutes, at which time the first appearance of a pink color develops in the test solution, is herewith suggested as a minimum standard requirement for medicinal mineral oils. Oils exhibiting such a peroxide test

value will be of high purity and stability, and, therefore, suitable for prolonged internal use.

SUMMARY

A lack of information concerning the expected stability and shelf life of mineral oils has been cited.

A method, whereby a prognosis can be made as to the stability of a given oil by a chemical determination of peroxides formed within the oil after heat treatment, is therefore suggested. This method is simple and requires little equipment.

A peroxide test of fifteen minutes is suggested as one of the minimum standards for medicinal mineral oils.

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Effect of Time and Temperature in the Preparation of Certain Fats and Waxes for Melting Point Determinations—Report No. Two*,†

By D. M. COPLEY‡

IN REPORT No. One (1) this Subcommittee brought to a conclusion its investigation of all official fats and waxes having melting point requirements, with the exception of cocoa butter which behaved erratically under the conditions of the United States Pharmacopœia melting-point methods (2). This second study is devoted to a series of cooperative melting-point determinations upon pressed and solvent extracted cocoa butter, all laboratories using four exactly comparable samples as distributed to them from a central source.

This series was directed toward two objectives: to find conditions which would give more concordant results, and, if possible, to shorten the time which seems necessary to season molten samples before applying melting-poiht determinations.

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† A Project of the Combined Pharmaceutical Contact Committee of the American Drug Manufacturers Association and the American Pharmaceutical Manufacturers Association.
‡ Chairman of the Cooperating Subcommittee which is composed of the following members: F. J. Austin, William R. Warner and Co.; R. A. Cain, Sharp and Dohme, Inc.; S. H. Fox and C. L. Stump, Gelatin Products Corp.; E. C. Merrill, United Drug Company; W. J. Rice, Eli Lilly and Co.; Joseph Rosin, Merck and Co., Inc.; E. B. Vliet, Abbott Laboratories; L. A. Watt and F. D. Smith, Monsanto Chemical Co.

EXPERIMENTAL

The following procedures were applied. After receipt, the samples were allowed to stand at least three days at room temperature before using. Melting-point tubes were charged with the molten samples as specified in U. S. P. XII (2) and additional tubes were prepared by boring into the unmelted material. These latter samples were used for melting-point determinations without further treatment.

The remaining tubes, originally charged with molten cocoa butter samples, were subdivided into groups and treated variously as follows: refrigeration at 10° or lower for thirty minutes, then room temperature (65° to 75° F.) for twenty-four hours, forty-eight hours, and seventy-two hours, respectively. Other tubes were kept at a temperature of −8° for one hour, two hours, three hours, and twenty-four hours. Upon the expiration of these time periods, melting points were determined by the U. S. P. XII method for Class II materials (2). One refinement rather widely tried consisted of the use of chemically clean melting-point tubes. There is considerable difference of opinion as to whether this improved the sharpness of the point of rise. Apparently it had no pronounced effect.

The findings of the various laboratories were accumulated in tabular form and appear in Tables I to VIII. A summary illustrating the concordance of the various determinations is included in Table IX.

TABLE I.—COCOA BUTTER, PRESSED, SAMPLE 1, SERIES 1

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Tubes filled cold	34.8	35.0	33.5	33.3	33.7	33.8	33.6	33.8	34.5	33.5
	34.8	35.0	33.6	33.4	33.8		33.5	33.9	35.0	
Chilled 30 min.	33.3	35.0	33.0	32.8	32.6	27.4	33.5	34.5	33.0	
Room temp. 24 hr.[a]	33.3	35.0	33.0	32.9	32.3	27.4	33.6	34.3	33.0	33.0
						30.5				
						31.0				
Chilled 30 min.	33.3	34.5	33.0	33.0	32.8	31.6	33.5	34.5	32.5	
Room temp. 48 hr.	33.3	35.0	33.1	33.1	32.5	30.6	33.5	34.3	32.5	33.0
						27.7				
						27.7				
Chilled 30 min.	33.3	35.0	33.1	33.1	33.3	32.0	33.5	34.5	32.0	
Room temp. 72 hr.	33.4	35.0	33.0	33.0	32.5	31.8	33.5	34.6	33.0	33.0

[a] Room temperature 65–75° F.

TABLE II.—COCOA BUTTER, PRESSED, SAMPLE 2, SERIES 1

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Tubes filled cold	34.0	35.5	33.3	33.2	33.8	34.1	33.6	33.7	34.0	
	34.0	36.0	33.2	33.1	33.5		33.6	33.6	34.0	33.5
Chilled 30 min.	32.2	34.5	32.2	32.7	32.5	29.1	33.4	33.7	33.0	
Room temp. 24 hr.[a]	32.7	34.5	32.2	32.8	32.1	31.9	33.4	33.6	32.0	32.5
						31.4				
						30.8				
Chilled 30 min.	32.5	34.0	32.2	32.6	32.5	30.7	33.4	33.8	33.0	
Room temp. 48 hr.	33.0	34.0	32.2	32.6	32.1	28.7	33.4	33.6	32.5	32.5
						31.4				
						31.4				
Chilled 30 min.	33.0	34.0	32.2	32.9	32.4	31.8	33.4	33.7	32.5	
Room temp. 72 hr.	33.0	34.0	32.4	32.8	32.1	32.0	33.2	33.6	32.5	32.5

[a] Room temperature 65–75° F.

TABLE III.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 1, SERIES 1

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Tubes filled cold	33.7	35.0	33.0	33.8	33.7	34.2	33.8	33.6	34.0	
	33.7	35.0	33.0	33.6	33.5		33.8	33.7	34.5	34.0
Chilled 30 min.	32.3	35.0	32.1	32.8	32.8	27.4	33.2	34.1	33.0	
Room temp. 24 hr.[a]	32.3	35.0	32.1	32.8	32.5	27.4	33.4	34.0	32.5	33.0
						27.8				
						30.6				
Chilled 30 min.	32.2	34.5	32.3	32.8	32.6	31.6	33.2	33.9	32.5	
Room temp. 48 hr.[a]	32.2	34.0	32.3	32.8	32.5	27.7	33.2	34.2	33.0	33.0
						31.7				
Chilled 30 min.	32.3	35.0	32.4	32.9	32.9	31.8	33.2	34.2	32.5	
Room temp. 72 hr.[a]	32.3	35.0	32.4	32.8	32.5	31.9	33.2	34.2	33.0	33.0

[a] Room temperature 65–75° F.

TABLE IV.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 2, SERIES 1

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Tubes filled cold	33.8	35.0	33.0	33.4	33.5	34.4	33.8	33.7	34.5	
	33.8	35.0	33.0	33.3	33.6		33.5	33.7	34.0	34.0
Chilled 30 min.	32.1	34.5	32.2	32.7	32.8	27.4	33.2	33.8	32.0	
Room temp. 24 hr.[a]	32.1	34.5	32.1	32.6	33.0	27.4	33.4	34.1	32.0	33.0
						27.8				
						30.5				
Chilled 30 min.	32.3	34.0	32.2	32.6	32.8	32.4	33.2	34.2	33.0	
Room temp. 48 hr.[a]	32.3	34.0	32.2	32.6	32.8	32.4	33.4	34.0	33.0	33.0
						29.6				
						31.7				
Chilled 30 min.	32.5	35.0	32.4	32.8	33.3	31.8	33.2	34.3	32.0	
Room temp. 72 hr.[a]	32.5	35.0	32.2	32.7	33.2	31.8	33.4	33.9	33.0	33.0

[a] Room temperature 65–75° F.

TABLE V.—COCOA BUTTER, PRESSED, SAMPLE 1, SERIES 2

	Laboratory									
	A	B	C	D[a]	E	F	G	H	I	J
Supercooled 1 hr.[b]	29.0	29.0	31.2	31.8	32.0	27.0	25.0	27.6	31.0	
	29.0	29.0	31.0	31.8	32.1	26.9	25.2	26.8	32.0	33.0
						26.1				
						26.5				
Supercooled 2 hr.[b]	28.0	29.0	31.0	31.5	32.2	26.8	25.0	27.3	30.0	
	28.0	29.0	30.9	31.7	32.4	26.8	25.0	27.6	30.0	33.0
						26.0				
						26.0				
Supercooled 3 hr.[b]	32.6	29.0	28.6	31.3	32.2	26.6	31.2	27.7	32.0	
	32.6	29.0	28.5	31.3	32.3	26.6	31.2	28.6	31.5	33.0
						27.6				
						27.8				
Supercooled 24 hr.[b]	32.5	29.0	31.8	32.1	32.3	26.3	30.6	31.5	29.0	
	32.5	29.0	31.8	32.0	32.3	26.2	30.8	28.5	30.0	33.0
						25.7				
						25.7				

[a] Fat transferred from −8° to 25° melts promptly. Tubes allowed to stand 20 min.
[b] Cooled to approximately −8°.

TABLE VI.—COCOA BUTTER, PRESSED, SAMPLE 2, SERIES 2

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Supercooled 1 hr.[a]	32.0	28.0	30.2	31.4	32.0	26.4	24.5	27.6	31.0	
	32.0	28.0	30.0	31.4	31.8	26.4	24.6	31.7	31.5	32.4
						26.2				
						26.3				
Supercooled 2 hr.[a]	31.6	28.0	30.6	31.4	31.5	26.4	30.5	27.0	31.5	
	31.6	28.0	30.6	31.2	31.9	26.4	25.0	30.4	31.5	32.6
						26.1				
						26.2				
Supercooled 3 hr.[a]	32.0	31.0	30.2	31.2	32.0	27.0	31.0	31.8	31.5	
	32.0	28.0	30.3	31.2	32.1	27.0	31.2	31.5	31.5	32.5
						30.4				
						30.4				
Supercooled 24 hr.[a]	32.2	32.0	31.2	31.3	32.1	30.6	30.5	32.5	30.0	
	32.2	32.0	31.3	31.2	32.2	30.7	30.5	32.3	29.0	32.5
						30.7				
						30.7				

[a] Cooled to approximately −8°.

TABLE VII.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 1, SERIES 2

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Supercooled 1 hr.[a]	28.1	29.0	31.2	31.3	31.0	27.1	25.0	23.0	31.0	
	28.2	30.0	31.4	31.3	31.5	27.1	24.8	26.8	31.0	33.0
						26.4				
						26.4				
Supercooled 2 hr.[a]	32.8	29.0	31.0	31.2	31.8	26.9	25.4	32.5	32.0	
	32.9	30.0	31.1	31.4	31.5	26.9	30.6	31.0	32.0	33.0
						26.3				
						26.1				
Supercooled 3 hr.[a]	32.5	32.0	31.0	31.3	31.8	26.8	25.4	31.3	32.0	
	32.1	30.0	31.0	31.3	31.2	26.7	25.4	31.5	32.0	33.0
						30.8				
						30.9				
Supercooled 24 hr.[a]	33.0	32.0	31.2	31.9	31.7	26.4	30.4	32.0	31.0	
	32.6	30.0	31.2	31.6	31.5	26.4	30.8	30.7	30.0	33.0
						25.7				
						25.7				

[a] Cooled to approximately −8°.

As in the first investigation, one cooperating laboratory extended the work to include congealing point determinations, plotting the time and temperature curves. This is undoubtedly an improvement over the usual method of reading the highest temporarily constant point only. Copies of these curves are included in this report as a matter of interest. (See Figs. 1, 2, 3, and 4.) However, since this study is devoted to melting-point conditions, no conclusions regarding the congealing properties were attempted but the data are valuable for reference purposes and for future consideration.

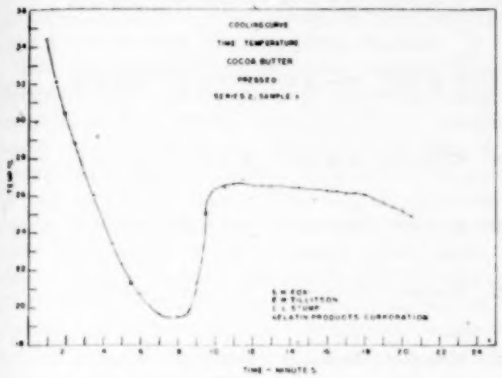


Figure 1

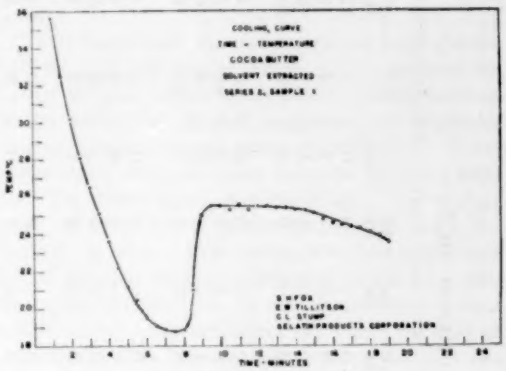
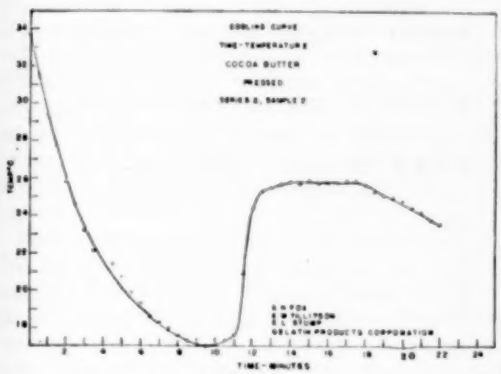


Figure 3



Fgiure 2

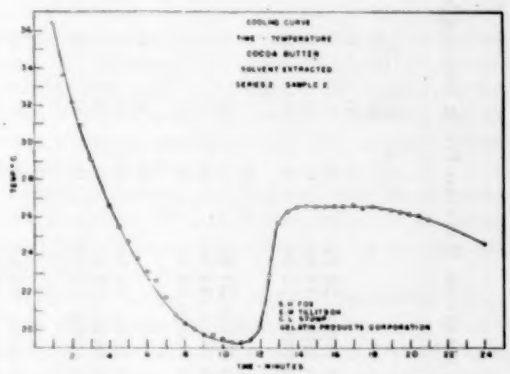


Figure 4

TABLE VIII.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 2, SERIES 2

	Laboratory									
	A	B	C	D	E	F	G	H	I	J
Supercooled 1 hr.[a]	28.0	29.0	31.2	31.4	26.0[b]	27.1	25.4	27.8	31.5	
	28.2	30.0	31.4	31.5	28.0[b]	27.1	25.5	31.2	32.0	32.9
						26.5				
						26.4				
Supercooled 2 hr.[a]	32.7	29.0	31.4	31.4	23.5[c]	26.7	31.5	27.3	32.0	
	33.2	30.0	31.4	31.3	26.0[b]	26.7	31.5	27.3	31.5	33.0
						26.0				
						26.0				
Supercooled 3 hr.[a]	32.2	33.0	31.4	31.2	23.0[c]	26.6	25.0	32.0	32.5	
	32.0	31.0	31.2	31.3	23.0[c]	26.6	25.2	30.7	31.5	32.9
						30.3				
						30.8				
Supercooled 24 hr.[a]	33.0	32.0	31.0	31.9	31.8	25.8	25.0	32.4	29.0	
	32.8	32.0	29.8	31.9	32.1	25.9	25.2	32.1	30.0	33.0
						25.7				
						25.8				

[a] Cooled to approximately −8°.
[b] Not true melting points—only a portion of the fat melted and rose.
[c] Not melting points. Temperature of bath. Fats rose at once.

TABLE IX.—SUMMARY OF MELTING-POINT DETERMINATIONS. REPORT NO. 2

		Below 25°	25°–26°	26°–27°	27°–28°	28°–29°	29°–30°	30°–31°	31°–32°	32°–33°	33°–34°	34°–35°	35°–36°	36° plus
Cocoa Butter, Pressed, No. 1	Tubes filled cold	0	0	0	0	0	0	0	0	0	12	3	3	0
	Chilled 30 min., room temp. 24 hr.	0	0	0	2	0	0	1	1	4	9	2	2	0
	Chilled 30 min., room temp. 48 hr.	0	0	0	2	0	0	1	1	4	9	3	1	0
	Chilled 30 min., room temp. 72 hr.	0	0	0	0	0	0	0	1	3	11	2	2	0
Cocoa Butter, Pressed, No. 2	Tubes filled cold	0	0	0	0	0	0	0	0	0	13	3	1	1
	Chilled 30 min., room temp. 24 hr.	0	0	0	0	0	1	1	2	10	5	2	0	0
	Chilled 30 min., room temp. 48 hr.	0	0	0	0	1	0	1	2	9	6	2	0	0
	Chilled 30 min., room temp. 72 hr.	0	0	0	0	0	0	0	1	10	6	2	0	0
Cocoa Butter, Solvent Extracted, No. 1	Tubes filled cold	0	0	0	0	0	0	0	0	0	14	2	2	0
	Chilled 30 min., room temp. 24 hr.	0	0	0	3	0	0	1	0	9	4	2	2	0
	Chilled 30 min., room temp. 48 hr.	0	0	0	1	0	0	0	2	9	5	3	0	0
	Chilled 30 min., room temp. 72 hr.	0	0	0	0	0	0	0	2	9	4	2	2	0
Cocoa Butter, Solvent Extracted, No. 2	Tubes filled cold	0	0	0	0	0	0	0	0	0	14	2	2	0
	Chilled 30 min., room temp. 24 hr.	0	0	0	3	0	0	1	0	9	5	3	0	0
	Chilled 30 min., room temp. 48 hr.	0	0	0	0	0	1	1	1	10	4	4	0	0
	Chilled 30 min., room temp. 72 hr.	0	0	0	0	0	0	0	2	7	7	1	2	0
Cocoa Butter, Pressed, No. 1	Supercooled 1 hr.	0	2	4	2	0	4	0	5	3	1	0	0	0
	Supercooled 2 hr.	0	2	4	2	2	2	1	5	2	1	0	0	0
	Supercooled 3 hr.	0	0	2	3	3	2	0	5	5	1	0	0	0
	Supercooled 24 hr.	0	2	2	0	1	3	3	3	6	1	0	0	0
Cocoa Butter, Pressed, No. 2	Supercooled 1 hr.	2	0	4	1	2	0	2	6	4	0	0	0	0
	Supercooled 2 hr.	0	1	4	1	2	0	4	8	1	0	0	0	0
	Supercooled 3 hr.	0	0	0	2	1	0	4	9	5	0	0	0	0
	Supercooled 24 hr.	0	0	0	0	0	1	7	4	9	0	0	0	0
Cocoa Butter, Solvent Extracted, No. 1	Supercooled 1 hr.	2	1	3	2	2	1	1	8	0	1	0	0	0
	Supercooled 2 hr.	0	1	4	0	0	1	2	7	5	1	0	0	0
	Supercooled 3 hr.	0	2	2	0	0	0	2	8	6	1	0	0	0
	Supercooled 24 hr.	0	2	2	0	0	0	5	7	3	2	0	0	0
Cocoa Butter, Solvent Extracted, No. 2	Supercooled 1 hr.	0	2	3	3	3	1	1	6	2	0	0	0	0
	Supercooled 2 hr.	1	0	5	2	0	1	1	7	2	2	0	0	0
	Supercooled 3 hr.	2	2	2	0	0	0	3	6	5	1	0	0	0
	Supercooled 24 hr.	0	6	0	0	0	2	1	4	6	2	0	0	0

DISCUSSION

The possibility of saving time by supercooling the melting-point samples for relatively short periods of time, or even including a twenty-four hour period, is not supported by the findings. The results are widely scattered, and while there is a concentration of comparable determinations, there are so many exceptions that the method cannot be considered properly applicable. One laboratory tried chilling with dry ice without significant improvement.

The four series wherein the molten samples were chilled for thirty minutes and held at a defined room temperature for varying time periods gave much better results. There was less divergence both in extent and numerically, and a better concentration of comparable findings. However, a pertinent number of determinations extended over a range of three degrees, and in one instance four degrees, which is a large portion of the total official tolerance. There was little choice between these aging periods. This method is the best to date starting with molten samples, but still fails to give a suitable degree of uniformity in results obtained.

The best results in three series were obtained when the melting-point tubes were charged with solid cocoa butter as received. The great majority of findings fell within one degree, 33° to 34°, and with one exception the number of determinations falling outside of a two-degree range were not sufficient to condemn the method. On the basis of this rather limited work the step indicates possibilities, even better than a similar series given in Report No. One. Perhaps with experience, technique is improving.

There are undoubtedly drawbacks to the method. The hardness of the cocoa butter at the time of sampling may affect the degree of contact between the sample and the melting-point tube. This is not very serious with cocoa butter but was found to be quite a factor with harder fats. The chief difficulty lies in the fact that cocoa butter may be received in molten form, or melting-point determinations may be desired during production or processing, starting with molten samples. In addition, it makes no provision for representative sampling.

Consequently some provision must be made for starting with melted material, as it is an inadequate method which does not cover all normal variations. This investigation throughout has been influenced by the U. S. P. requirement of rapid chilling of the melted samples. Evidence is accumulating to indicate that this practice may be harmful rather than helpful.

There have been various comments from the cooperating laboratories pointing out the unusual behavior of cocoa butter under conditions encountered in this work (3). It was suggested that originally molten samples should stand at about 75° F. for about three days, charging the tube with the solid fat. An observation was made that the surface crust of solid cocoa butter was firmer than the underlying layer. One laboratory separated cocoa butter into an oily fluid fraction and a hard fraction by crystallization from isopropyl alcohol at room temperature. Another laboratory which has worked extensively with cocoa butter pointed out that the melted fat, upon cooling, may assume one of two or possibly three crystalline forms, melting at about 26°, 32°, and 34°, respectively.

This laboratory suggested that the melting-point tubes, after charging with the melted fat, be held at 18 to 20° for sixteen to twenty-four hours or, better, that the melted fat be brought to 18° to 20°, stirring until it begins to solidify, and after standing a short time, charging the tubes with the hardened cocoa butter.

Buchi and Oesch (4) recorded that the temperature of melting of cocoa butter influenced its solidification and melting point, and that stirring affected these points somewhat.

This new factor, that different crystalline structures which affect the melting points may be obtained, means that conditions must be devised which will always result in the same structure for the purpose of melting-point standards. Preliminary trials utilizing the above suggestions indicate that this may be done, although the work must be extended considerably to establish the point.

CONCLUSIONS AND SUMMARY

1. Cooperative studies upon the conditions for melting-point determinations upon cocoa butter have been continued.

2. Extreme chilling of the molten samples for periods of one hour to twenty-four hours did not result in proper concordance of results for control purposes.

3. Thirty minutes' chilling followed by varying intervals up to three days at 65° to 75° F. improved concordances greatly, but there were enough exceptions to cast doubt upon the procedure as a standard method.

4. The best results were obtained by charging the melting-point tubes with solid cocoa butter by boring into the cake. However, this makes no provision for material received in softened or molten condition, nor does it permit representative sampling.

5. The accumulated evidence indicates that the unusual behavior of cocoa butter under the preparatory conditions of the U. S. P. melting-point method is probably due to

varying crystalline structures formed during cooling. Rapid chilling now appears inadvisable.

6. Based on the above, the development of better preparatory conditions for this determination seems possible.

REFERENCES

(1) Copley, D. M., THIS JOURNAL, 34, 165–70(1945).
(2) "United States Pharmacopœia," Twelfth Revision, Mack Printing Company, Easton, Pa., 1942, p. 595.
(3) Reports and communications of the cooperating laboratories.
(4) Buchi, J., and Oesch, P., "Melting and solidification of cocoa butter," "Hundert Jahre Schweiz. Apoth.-Ver. (Centenaire Soc. Suisse Pharm.) 1843–1943," 1943, pp. 333–41.

Effect of Time and Temperature in the Preparation of Certain Fats and Waxes for Melting-Point Determinations—Report No. Three*,†

By D. M. COPLEY‡

IN REPORT No. One of this Subcommittee, melting-point conditions for official fats and waxes were studied (1), and in Report No. Two the study was continued and a special investigation of cocoa butter carried out (2). This latter investigation required still further trials which are completed in this series No. Three.

For cooperative tests, six samples of cocoa butter, three prepared by pressing and three by solvent extraction, were subdivided to provide as nearly as possible identical samples. In preparing these samples, each unit was melted and stirred thoroughly until cool prior to subdivision. One set of samples was forwarded to each Subcommittee member.

EXPERIMENTAL

The following procedures were applied. After receipt the samples were allowed to stand at least three days at 70° F. (21° C.) or somewhat lower. Then melting-point tubes were charged by boring into the samples and melting points determined as in previous investigations.

Then each sample was melted at 50°, mixed well and approximately 15 Gm. of each transferred to standard 100-cc. beakers. The beakers were immediately placed at a temperature of 18° to 22° and stirred continuously until congealed, the completion of stirring being reached when the material held its shape. The beakers were kept within the above temperature range during the entire test period.

After one hour, melting-point tubes were charged by boring into the solid samples and melting-point determinations run following the U. S. P. XII method for Class II materials (3) with the exception of the preparation of the samples. The same procedure was repeated after the samples had stood two hours, four hours, and overnight. The results were accumulated and appear in the Tables I to VI. Also in Table VII appears a summary illustrating the concordance of the various findings.

DISCUSSION

It is evident that the conditions used in this series of determinations give a much better degree of concordance than the previous trials reported. It is noted that by this method the melting point is somewhat depressed in relation to the determinations upon the original unmelted samples. The majority of findings fall between 32° and 33° for samples receiving the special treatment, while in the determinations upon the original unmelted samples, findings between 33° and 35° predominate. It is the opinion of this Subcommittee that the method here outlined does create a set of conditions which gives good concordance as a control method, recognizing that the findings are undoubtedly somewhat below the maximum melting points obtainable.

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† A Project of the Combined Pharmaceutical Contact Committee of the American Drug Manufacturers Association and the American Pharmaceutical Manufacturers Association.
‡ Chairman of the Cooperating Subcommittee which is composed of the following members: F. J. Austin, William R. Warner and Co.; R. A. Cain, Sharp and Dohme, Inc.; S. H. Fox and C. L. Stump, Gelatin Products Corp.; E. C. Merrill, United Drug Company; W. J. Rice, Eli Lilly and Co.; Joseph Rosin, Merck and Co., Inc.; E. B. Vliet, Abbott Laboratories; L. A. Watt and F. D. Smith, Monsanto Chemical Co.

TABLE I.—COCOA BUTTER, PRESSED, SAMPLE 1, SERIES 3

	Laboratory								
	A	B	C	D	E	F	G	H	I
Tubes filled cold from original sample	34.0 34.1 34.0 34.0	35.0 35.1	34.9 34.7	33.9 33.9	34.2	33.8 33.8	34.6 34.9	34.5 34.5	34.0
Melted 50°, solidified 18–22°, m. p. taken after 1 hr.	32.7 32.7	33.0 33.0	32.5 32.5	31.7 31.7	32.4	32.2 32.2 32.2 32.2	32.8 32.9	32.0 32.0	32.0
Same except m. p. taken after 2 hr.	32.7 32.7	32.5 33.0	32.2 32.4	31.8 31.8	32.4	32.0 32.0 32.1 32.1	32.8 32.8	32.5 32.2	32.5
Same except m. p. taken after 4 hr.	32.7 32.7	32.5 33.0	32.2 32.2	31.8 31.8	32.4	32.1 32.1	32.8 32.8	32.5 32.0	32.5
Same except m. p. taken next day.	32.9 32.9	32.5 33.0	32.4 32.4	31.8 31.8	32.4	32.1 32.4	32.9 33.0	32.5 32.5	32.5

TABLE II.—COCOA BUTTER, PRESSED, SAMPLE 2, SERIES 3

	Laboratory								
	A	B	C	D	E	F	G	H	I
Tubes filled cold from original sample	33.2 33.2 33.3 33.3	34.8 34.8	34.2 34.2	33.9 33.8	33.6	33.2 33.2	34.5 34.6	34.5 35.0	34.0
Melted 50°, solidified 18–22°, m. p. taken after 1 hr.	32.0 32.0	32.0 32.5	32.2 32.2	31.3 31.3	32.0	31.6 31.6 32.0 32.0	32.5 32.5	32.0 32.0	32.0
Same except m. p. taken after 2 hr.	32.0 32.0	32.0 32.5	32.0 32.0	31.3 31.4	32.0	31.5 31.5 31.7 31.7	32.4 32.4	32.5 32.5	32.0
Same except m. p. taken after 4 hr.	32.3 32.3	32.0 32.0	31.7 31.8	31.4 31.4	32.0	31.6 31.7 31.6 31.6	32.4 32.4	32.0 32.5	32.5
Same except m. p. taken next day.	32.6 32.6	32.0 32.0	31.7 31.7	31.4 31.4	32.2	31.6 31.6	32.6 32.5	32.5 32.5	32.5

TABLE III.—COCOA BUTTER, PRESSED, SAMPLE 3, SERIES 3

	Laboratory								
	A	B	C	D	E	F	G	H	I
Tubes filled cold from original sample	34.0 34.0 34.0 34.0	35.0 35.0	34.7 34.6	34.0 33.9	34.2	34.0 34.0	34.8 34.6	34.5 35.0	34.0
Melted 50°, solidified 18–22°, m. p. taken after 1 hr.	32.3 32.3	32.0 33.0	32.6 32.5	31.7 31.7	32.4	32.2 32.3 32.2 32.3	32.7 32.8	32.0 32.5	32.0
Same except m. p. taken after 2 hr.	32.3 32.3	32.5 33.0	32.2 32.3	31.6 31.7	32.4	31.8 31.8 32.1 32.3	32.9 32.8	33.0 32.5	32.5
Same except m. p. taken after 4 hr.	32.7 32.7 32.6 32.8	32.5 33.0	32.2 32.1	31.8 31.8	32.4	31.9 32.0 32.1 32.1	32.8 32.5	32.5 32.5	32.5
Same except m. p. taken next day.	32.3 32.4	32.5 32.5	32.2 32.2	31.7 31.8	32.4	32.1 32.1	33.0 32.8	33.0 32.5	32.5

TABLE IV.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 1, SERIES 3

	Laboratory								
	A	B	C	D	E	F	G	H	I
Tubes filled cold from original sample	33.5	36.0	34.8	40.0	34.0	33.5	33.9	36.0	34.0
	33.5	36.0	34.9	40.0		33.8	35.8	35.5	
	33.5								
	33.5								
Melted 50°, solidified 18–22°, m. p. taken after 1 hr.	32.1	32.0	32.2	31.5	32.0	31.3	32.4	32.5	32.0
	32.1	33.0	32.3	31.5		31.3	32.4	32.0	
						31.8			
						31.9			
Same except m. p. taken after 2 hr.	32.0	32.5	32.2	31.5	32.0	31.6	32.4	33.0	32.5
	32.0	32.5	32.2	31.5		31.6	32.4	32.5	
						31.7			
						31.7			
Same except m. p. taken after 4 hr.	32.1	33.0	31.7	31.5	32.0	31.2	32.4	32.5	32.5
	32.2	33.0	31.7	31.5		31.7	32.6	32.5	
						31.7			
						31.7			
Same except m. p. taken next day	32.6	32.5	32.2	31.7	32.2	31.5	32.5	32.0	33.0
	32.6	32.5	32.1	31.7		31.6	32.6	32.5	

TABLE V.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 2, SERIES 3

	Laboratory								
	A	B	C	D	E	F	G	H	I
Tubes filled cold from original sample	33.3	36.0	34.0	40.1	33.6	33.3	34.2	35.5	34.0
	33.3	36.0	34.1	40.0		33.3	34.3	35.5	
	33.3								
	33.3								
Melted 50°, solidified 18–22°, m. p. taken after 1 hr.	32.0	32.0	31.9	31.4	32.0	31.2	32.4	32.5	32.0
	33.0	32.0	32.0	31.5		31.3	32.6	32.5	
						31.8			
						31.8			
Same except m. p. taken after 2 hr.	32.0	32.5	32.0	31.5	31.8	31.6	32.8	32.5	32.5
	32.1	33.0	32.0	31.5		31.6	32.6	33.0	
						31.8			
						31.7			
Same except m. p. taken after 4 hr.	32.1	33.0	31.6	31.5	31.8	31.2	32.6	32.5	32.5
	32.2	33.0	31.5	31.6		31.8	32.5	32.5	
						31.7			
						31.6			
Same except m. p. taken next day	32.7	32.0	31.9	31.6	32.2	31.5	32.6	33.0	32.5
	32.8	32.5	31.8	31.6		31.5	32.6	33.0	

TABLE VI.—COCOA BUTTER, SOLVENT EXTRACTED, SAMPLE 3, SERIES 3

	Laboratory								
	A	B	C	D	E	F	G	H	I
Tubes filled cold from original sample	33.0	36.0	33.9	40.1	33.8	33.2	35.4	35.0	34.0
	33.0	36.0	33.9	40.1		33.2	35.9	35.0	
	33.1								
	33.2								
Melted 50°, solidified 18–22°, m. p. taken after 1 hr.	32.0	32.0	32.2	31.5	32.0	31.7	32.5	32.0	32.0
	32.0	33.0	32.2	31.4		31.7	32.4	32.0	
Same except m. p. taken after 2 hr.	32.0	32.5	32.0	31.4	32.0	31.6	32.6	32.5	32.5
	32.0	33.0	32.1	31.4		31.7	32.6	32.5	
						31.8			
						31.8			
Same except m. p. taken after 4 hr.	32.0	33.0	31.6	31.5	32.0	31.3	32.6	32.5	32.5
	32.0	33.0	31.8	31.5		31.3	32.8	33.0	
						31.6			
						31.7			
Same except m. p. taken next day	32.4	32.5	32.2	31.5	32.2	31.5	32.7	33.0	32.5
	32.4	32.5	32.0	31.6		31.6	32.7	32.5	

The concentration of melting points between 32° and 33° but with a substantial number between 31° and 32° is so conspicuous that the Chairman's laboratory became concerned lest an artificial condition were being created which would result in such findings, regardless of exact nature of the cocoa butter samples. Consequently a supply of cocoa butter was separated by solvent fractionation into a high-melting portion and a low-melting portion. These portions were individually combined with normal cocoa butter and the melting-point determinations repeated. It was found that this method of determination measured the differences in melting points created under these conditions. Consequently the method herein recommended does not set up artificial conditions giving false resul[illegible]

It is recognized that sampling by boring into the original hard cake of cocoa butter very probably gives a truer melting point. However, this is not good official procedure since it does not allow for representative sampling, or if cross-section samples are made by melting portions together in order to get

TABLE VII.—SUMMARY OF MELTING-POINT DETERMINATIONS, SERIES NO. 3

		Below 31°	31°–32°	32°–33°	33°–34°	34°–35°	35° and above
Cocoa Butter, Pressed, No. 1	Tubes filled cold from original sample	0	0	0	4	12	2
	Melted 50°, solidified 18–22°, m. p. after 1 hr.	0	2	14	2	0	0
	Melted 50°, solidified 18–22°, m. p. after 2 hr.	0	2	15	1	0	0
	Melted 50°, solidified 18–22°, m. p. after 4 hr.	0	2	15	1	0	0
	Melted 50°, solidified 18–22°, m. p. after overnight	0	2	12	2	0	0
Cocoa Butter, Pressed, No. 2	Tubes filled cold from original sample	0	0	0	9	8	1
	Melted 50°, solidified 18–22°, m. p. after 1 hr.	0	4	14	0	0	0
	Melted 50°, solidified 18–22°, m. p. after 2 hr.	0	6	12	0	0	0
	Melted 50°, solidified 18–22°, m. p. after 4 hr.	0	8	10	0	0	0
	Melted 50°, solidified 18–22°, m. p. after overnight	0	6	10	0	0	0
Cocoa Butter, Pressed, No. 3	Tubes filled cold from original sample	0	0	0	1	14	3
	Melted 50°, solidified 18–22°, m. p. after 1 hr.	0	2	15	1	0	0
	Melted 50°, solidified 18–22°, m. p. after 2 hr.	0	4	12	2	0	0
	Melted 50°, solidified 18–22°, m. p. after 4 hr.	0	3	16	1	0	0
	Melted 50°, solidified 18–22°, m. p. after overnight	0	2	12	2	0	0
Cocoa Butter, Solvent Extracted, No. 1	Tubes filled cold from original sample	0	0	0	7	4	5
	Melted 50°, solidified 18–22°, m. p. after 1 hr.	0	6	11	1	0	0
	Melted 50°, solidified 18–22°, m. p. after 2 hr.	0	6	11	1	0	0
	Melted 50°, solidified 18–22°, m. p. after 4 hr.	0	8	8	2	0	0
	Melted 50°, solidified 18–22°, m. p. after overnight	0	4	11	1	0	0
Cocoa Butter, Solvent Extracted, No. 2	Tubes filled cold from original sample	0	0	0	7	5	6
	Melted 50°, solidified 18–22°, m. p. after 1 hr.	0	7	10	1	0	0
	Melted 50°, solidified 18–22°, m. p. after 2 hr.	0	7	9	2	0	0
	Melted 50°, solidified 18–22°, m. p. after 4 hr.	0	9	7	2	0	0
	Melted 50°, solidified 18–22°, m. p. after overnight	0	6	8	2	0	0
Cocoa Butter, Solvent Extracted, No. 3	Tubes filled cold from original sample	0	0	0	9	1	8
	Melted 50°, solidified 18–22°, m. p. after 1 hr.	0	6	11	1	0	0
	Melted 50°, solidified 18–22°, m. p. after 2 hr.	0	6	11	3	0	0
	Melted 50°, solidified 18–22°, m. p. after 4 hr.	0	8	7	3	0	0
	Melted 50°, solidified 18–22°, m. p. after overnight	0	4	11	1	0	0

representative material, there is a long delay in waiting for the samples to return to normal. One laboratory found that in a checkup after thirty days' standing the original melting points were attained.

On the other hand, the method here described does permit cross-section sampling, and takes a relatively small amount of time. Various laboratories suggested time periods before taking the melting points ranging from one hour to overnight, but two hours seems to be quite acceptable. Consequently it is recommended that this method be presented for the official determination of the melting point of cocoa butter. If adopted the tolerance should be lowered one degree throughout in recognition of the fact that the method does give a somewhat depressed melting point.

CONCLUSIONS AND SUMMARY

1. Cooperative studies upon the conditions for melting-point determinations upon cocoa butter have been completed.

2. The creation of a standard crystalline structure by melting the sample of cocoa butter at 50°, immediately placing within a range of 18° to 22° and stirring until congealed, then sampling by boring into the solidified cake, results in concordant melting-point determinations.

3. These findings indicate a depression of melting point in comparison with samples taken from original solidified material, but the depression is a uniform factor.

4. The time element between preparing samples and taking melting points may be well set at two hours.

5. The method herein suggested permits representative sampling.

6. It is recommended that the U. S. P. Revision Committee give consideration to this method as an official standard.

7. If considered as an official method, the required melting-point range should be reduced one degree throughout.

8. The revised method should read approximately as follows: Melt a sample of not less than 15 Gm. of Cocoa Butter at a temperature of 50°. Mix well and transfer approximately 15 Gm. to a standard 100-cc. beaker. Place at once at a temperature of 18° to 22° and stir continuously until congealed, this point being determined when the material holds it shape. Retain the sample at this temperature for two hours. Charge the melting-point tube by boring into the solidified cake and proceed as under Melting Points for Class II Materials, beginning with "Then attach the tube to a standard thermometer" (p. 597 U. S. P. XII).

REFERENCES

(1) Combined Contact Committee Report, THIS JOURNAL, 34, 165(1945).
(2) Combined Contact Committee Report, THIS JOURNAL, 35, 78–84(1946).
(3) "United States Pharmacopœia," Twelfth revision, Mack Printing Company, Easton, Pa., 1942, p. 595.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

3-Nitro-1-naphthoic acid
Uridylic acid
1,8-Diamino naphthalene
2-Chloro-4-dimethylamino-6-methyl pyrimidine
Coniferyl alcohol
Sodium-4-methoxy salicylate
Ethylene dilaurate
5-Methyl-2-amino-1,3,4-thiodiazol
Cytochrome C
d- and *l*-Gulose
d- and *l*-Idose
l-Ribose
l-Lyxose
Allocinnamic acid
Isocrotonic acid
Muscarine
Clupeine
Salmine
Quinic acid

The Toxicity of Myristyl-gamma-Picolinium Chloride*

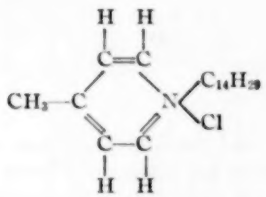
By JOHN W. NELSON and STANLEY C. LYSTER†

The local and systemic toxicity of myristyl-γ-picolinium chloride in rats, rabbits, and dogs together with limited comparative data on cetyl pyridinium chloride are reported. The conclusion is that a 1:1000 solution has a wide margin of safety.

THE preparation and germicidal activity of a series of alkyl halide quaternary ammonium compounds has been reported from this Laboratory by Kolloff, Wyss, Himelick, and Mantele (1). Huyck (2) has reviewed the literature concerning the type of compound with special reference to mode of action, relationship of chemical constitution to germicidal activity, and pharmacological actions.

We wish to report the results of local and systemic toxicity studies with the single member of the series, myristyl-γ-picolinium chloride, which has interesting possibilities as a superior germicide. For purposes of comparison limited parallel studies have been made using cetyl pyridinium chloride.

Myristyl-γ-picolinium chloride is a crystalline, cationic, germicidal substance exhibiting marked detergent action. Its chemical structure is:



It is soluble in water, alcohol, and acetone. A 1–1000 solution in physiological saline is colorless, odorless, stable, and has a soapy feel.

EXPERIMENTAL

Acute Toxicity in Rats.—Healthy male rats of a Sprague–Dawley strain, raised in our own animal colony, were selected in a weight range of 175 to 370 Gm. although a majority of the animals weighed 200 to 300 Gm. The rats were maintained in an air-conditioned room with an average temperature of 78° F. and had free access to water and Purina Dog Chow at all times.

Parallel determinations of the minimum lethal doses of myristyl-γ-picolinium chloride and cetyl pyridinium chloride were made intravenously, intraperitoneally, subcutaneously, and by stomach tube. A 10-mg./ml. solution in physiological saline was used intravenously and intraperitoneally. A 30-mg./ml. solution was used subcutaneously and orally. The LD_{50} is the smallest dose of the substance killing 50% of the treated animals. Cetyl pyridinium chloride, Batch No. E-1328-3, was purchased from the Edwal Laboratories, Chicago.

The results of the acute toxicity experiments with myristyl-γ-picolinium chloride are summarized in Table I and cetyl pyridinium chloride in Table II. All of the animals were observed for delayed deaths for a period of twenty-two to forty-two days after injection.

Table I indicates that the approximate LD_{50}'s of myristyl-γ-picolinium chloride are 200 mg./Kg. subcutaneously, 7.5 mg./Kg. intraperitoneally, 30 mg./Kg. intravenously, and 250 mg./Kg. by stomach tube. The corresponding values from Table II for cetyl pyridinium chloride are 250 mg./Kg. subcutaneously, 6 mg./Kg. intraperitoneally, 30 mg./Kg. intravenously, and 200 mg./Kg. by stomach tube. Thus, myristyl-γ-picolinium chloride is slightly less toxic than cetyl pyridinium chloride intraperitoneally or orally and slightly more toxic subcutaneously. The intravenous toxicities are approximately equal.

Fatal doses of myristyl-γ-picolinium chloride intravenously or subcutaneously cause extensive tissue damage. After intravenous injection there was marked cyanosis and necrosis which in a few instances caused loss of the tail. Denudation of the fur at the site of administration usually occurs twelve to twenty-four hours after subcutaneous injection of large doses of the 3% solution. This is followed by sloughing of the skin and necrosis of the underlying tissue. Large oral doses of the 3% solution caused erosion of the gastric mucosa. Higher doses of the 1% solution administered intraperitoneally produced inflammation. With fatal doses a curare-like action was observed with peripheral paralysis and central nervous stimulation. All of these toxic effects observed with myristyl-γ-picolinium chloride were also observed to an equal degree with cetyl pyridinium chloride.

Rats receiving the lower doses of the germicide lost weight for a few days then gained in weight until the end of the experiment.

* Received Oct. 1, 1945, from the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.
† Acknowledgment is made to Dr. William F. Wenner and Dr. Walter Schiller for the gross and microscopic examination of the tissues.

TABLE I.—ACUTE TOXICITY OF MYRISTYL-γ-PICOLINIUM CHLORIDE IN RATS

Dose Injected (Mg./Kg.)	Subcutaneous		Intraperitoneal		Intravenous		Stomach Tube	
	No. of Rats Treated	% Mortality	No. of Rats Treated	% Mortality	No. of Rats Treated	% Mortality	No. of Rats Treated	% Mortality
800	..	...	..	...	..	..	5	100
600	..	...	..	...	..	..	6	100
400	5	100	..	...	..	..	5	100
300	5	80	..	...	..	..	..	...
250	..	...	..	...	..	..	10	50
200	15	93	..	...	..	..	15	40
175	10	20	..	...	..	..	10	10
150	9	11	..	...	..	..	5	0
100	5	0	..	...	..	..	5	0
30	..	...	5	100	15	87	..	...
25	..	...	5	100	15	20	..	...
20	..	...	10	100	15	27	..	...
15	..	...	15	93	5	0	..	...
12.5	..	...	10	80	..	..	..	...
10	..	...	25	64	..	..	..	...
7.5	..	...	10	50	..	..	..	...
5	..	...	10	30	..	..	..	...
2.5	..	...	10	0	..	..	..	...
Approx. LD_{50} (mg./Kg.)	200		7.5		30		250	

TABLE II.—ACUTE TOXICITY OF CETYL PYRIDINIUM CHLORIDE IN RATS

Dose Injected (Mg./Kg.)	Subcutaneous		Intraperitoneal		Intravenous		Stomach Tube	
	No. of Rats Treated	% Mortality	No. of Rats Treated	% Mortality	No. of Rats Treated	% Mortality	No. of Rats Treated	% Mortality
450	10	60	..	...	..	..	10	70
400	13	70	..	...	..	..	13	77
350	10	70	..	...	..	..	10	60
300	23	48	..	...	..	..	13	77
250	20	50	..	...	..	..	10	60
200	23	39	..	...	..	..	13	54
100	3	0	..	...	..	..	3	0
30	..	...	3	100	13	54	..	...
25	..	...	13	92	13	0	..	...
20	..	...	13	92	13	0	..	...
15	..	...	13	85	3	33	..	...
12.5	..	...	10	100	..	..	..	...
10	..	...	10	90	..	..	..	...
7.5	..	...	10	100	..	..	..	...
5	..	...	10	40	..	..	..	...
2.5	..	...	10	30	..	..	..	...
Approx. LD_{50} (mg./Kg.)	250		6		30		200	

Chronic Toxicity in Rats.—For the chronic toxicity experiments 65 male Sprague–Dawley strain rats were used. These were forty-seven to fifty-seven days of age and weighed 141 to 180 Gm. at the time of the first administration of the germicide. The diet consisted of finely ground Purina Dog Chow. Body weights and food and water consumption were recorded daily.

Myristyl-γ-picolinium chloride was administered daily for sixty days or until death of the animals, to groups of five rats each, at levels of 0.25, 0.10, 0.05, and 0.025% of the diet. In another series the sole source of drinking water was 0.25, 0.10, 0.05, and 0.025% solutions of the germicide. A third series received 100, 40, 10, and 5 mg./Kg. body weight once daily by stomach tube. A fourth group of five control rats received only diet and water *ad lib.* The average growth, food, and water consumption and calculated average daily doses of the germicide per kilogram of body weight are summarized for each successive ten-day interval in Table III. Growth curves, plotted at five-day intervals for the various groups, are given in Figs. 1, 2, and 3.

Table III indicates that myristyl-γ-picolinium chloride when fed as 0.025, 0.05, and 0.10% of the diet permits normal growth. The average gains over the entire period were 130, 124, and 122 Gm., respectively, per rat as compared to 137 Gm. for the controls. These doses correspond to approximately 17, 36, and 60 mg./Kg. per day, respectively. However, at the level of 0.25% of the diet, corresponding to 139 mg./Kg. per day, growth was definitely retarded since the average gain was only 43 Gm. per rat for the sixty-day period.

When a 0.25% solution of the drug served as the

TABLE III.—CHRONIC TOXICITY OF MYRISTYL-γ-PICOLINIUM CHLORIDE

Period		Controls Untreated	Drug Mixed with Diet 0.25%	0.10%	0.05%	0.025%	Drug Mixed with Drinking Water 0.25%	0.10%	0.05%	0.025%	Drug Given by Stomach Tube 100 Mg./Kg.	40 Mg./Kg.	10 Mg./Kg.	5 Mg./Kg.
First 10 days	Initial Body Weight (av.), Gm.	165.4	158.2	160.0	161.0	147.2	156.0	159.4	154.4	147.2	164.6	166.2	157.4	150.6
	Weight Gain (av.), Gm.	39.4	17.8	28.0	39.6	44.0	59.4	38.0	14.2	21.7	32.1	29.3	19.4	36.6
	Av. Food Consumed/Rat/Day, Gm.	15.4	9.5	12.9	13.9	13.0	4.7	8.4	8.5	10.6	7.0	12.7	15.0	13.5
	Av. Water Consumed/Rat/Day, cc.	28.9	16.8	20.9	25.8	25.9	7.3	10.3	15.8	20.3	23.6	23.4	24.6	27.0
	Av. Dose of Drug/Kg./Day, mg.	0.0	160.0	74.0	38.4	20.0	144.4	72.5	49.0	32.1	100.0	40.0	10.0	5.0
Second 10 days	Av. Weight Gain, Gm.	32.0	1.0	28.6	30.1	29.6	...	0.0	28.2	19.9	...	16.2	28.8	33.6
	Av. Food Consumed/Rat/Day, Gm.	19.5	9.2	13.4	17.3	15.4	...	6.4	10.5	11.9	...	9.4	16.7	16.7
	Av. Water Consumed/Rat/Day, cc.	33.0	16.6	27.7	26.1	27.8	...	9.0	22.1	23.3	...	32.8	29.5	32.7
	Av. Dose of Drug/Kg./Day, mg.	0.0	164.3	66.4	40.0	18.7	Dead	...	60.5	32.5	Dead	40.0	10.0	5.0
Third 10 days	Av. Weight Gain, Gm.	8.0	29.8	28.2	26.3	23.6	...	...	22.6	20.4	...	...	29.0	4.8
	Av. Food Consumed/Rat/Day, Gm.	17.2	9.2	14.4	18.1	16.8	...	...	14.4	12.4	...	...	17.6	15.1
	Av. Water Consumed/Rat/Day, cc.	32.3	20.9	29.1	42.5	30.4	...	...	25.0	25.9	...	...	30.0	33.6
	Av. Dose of Drug/Kg./Day, mg.	0.0	148.4	62.4	37.1	18.0	...	Dead	60.0	32.5	...	Dead	10.0	5.0
Fourth 10 days	Av. Weight Gain, Gm.	14.6	9.8	20.8	7.0	15.8	...	...	21.8	24.0	...	...	30.5	13.0
	Av. Food Consumed/Rat/Day, Gm.	17.0	9.3	15.9	16.6	16.9	...	...	12.7	17.4	...	...	15.7	15.5
	Av. Water Consumed/Rat/Day, cc.	34.0	21.0	30.6	44.2	29.5	...	...	24.8	27.2	...	...	30.5	32.0
	Av. Dose of Drug/Kg./Day, mg.	0.0	133.0	62.4	38.0	16.7	...	...	53.3	30.6	...	...	10.0	5.0
Fifth 10 days	Av. Weight Gain, Gm.	26.6	21.0	16.6	19.8	17.4	...	...	12.4	12.8	...	...	30.5	28.8
	Av. Food Consumed/Rat/Day, Gm.	18.4	9.5	13.4	17.5	16.6	...	...	12.9	16.5	...	...	16.4	19.4
	Av. Water Consumed/Rat/Day, cc.	37.8	22.2	30.5	33.9	31.8	...	...	24.1	28.2	...	...	31.7	37.9
	Av. Dose of Drug/Kg./Day, mg.	0.0	125.0	49.0	31.8	15.3	...	...	48.7	29.8	...	...	10.0	5.0
Sixth 10 days	Av. Weight Gain, Gm.	22.0	0.0	9.2	14.5	3.6	...	...	14.4	11.6	...	...	17.3	22.6
	Av. Food Consumed/Rat/Day, Gm.	18.6	8.4	14.0	18.6	17.4	...	...	13.4	17.5	...	...	16.4	19.7
	Av. Water Consumed/Rat/Day, cc.	36.3	23.8	29.7	34.2	30.4	...	...	22.8	28.2	...	...	31.9	43.1
	Av. Dose of Drug/Kg./Day, mg.	0.0	1050.	49.0	31.8	15.4	...	...	42.6	27.8	...	...	10.0	5.0
	Final Body Weight, Gm.	302.0	201.0	282.2	284.5	277.6	...	...	268.0	259.6	...	...	283.0	284.8

sole source of drinking water an average loss in body weight of 59 Gm. occurred during the first ten-day period and all of the rats were dead before the fifteenth day of the experiment. Likewise, the 0.10% solution was toxic and the animals died in

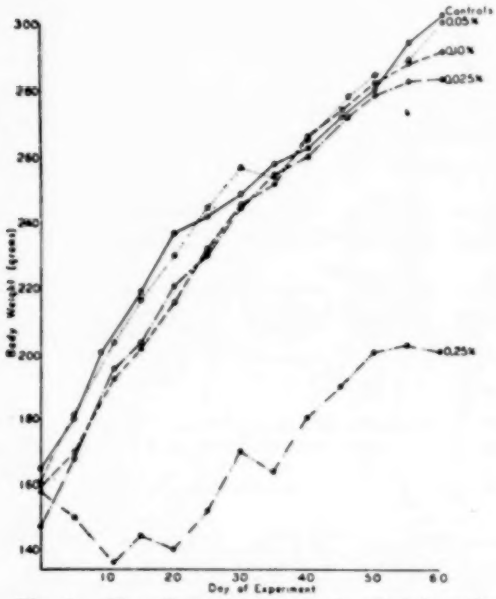


Fig. 1.—The effect of the chronic administration of myristyl γ-picolinium chloride mixed with the diet on the body weight of rats.

sixteen to twenty-seven days. However, the 0.05 and 0.025% solutions, corresponding to 52 and 31 mg./Kg. per day, were better tolerated and permitted average growths of 114 and 112 Gm., respectively.

Myristyl-γ-picolinium chloride given by stomach tube once daily, at doses of 100 and 40 mg./Kg., was toxic and caused death of all animals before the twenty-first day. The 10- and 5-mg./Kg. doses permitted normal growth of 126 and 130 Gm., respectively. That the germicide appears to be more toxic by stomach tube than by mixing with the diet or in the drinking water is probably due to the fact that the full daily dose was administered at one time. If repeated small doses had been given the animals would undoubtedly tolerate larger amounts of the drug.

Chronic Toxicity Orally in Dogs.—One female and three male liter mate mongrel pups approximately three months of age, weighing 2200 to 2500 Gm. at the start of the experiment, were given 0, 5, 10, and 20 mg. myristyl-γ-picolinium chloride per kilogram body weight once daily for ninety days. The daily dose in aqueous solution was added to the morning meal. The diet consisted of fresh ground horse meat and Miller's Dog Kibbles supplemented with vitamins and minerals. Body weights were recorded daily, but for ease of presentation, these data are plotted at five-day intervals in Fig. 4.

The dogs were sacrificed on the ninety-first day when gross and microscopic examination of the tissues were made. The microscopic examination revealed normal lungs, stomach, large and small intestines, urinary bladder, myocardium, aorta, carotid, coronary arteries, liver, adrenals, spleen, and lymph nodes. The liver showed minimal to marked glycogen storage with no evidence of fatty infiltration. The kidney stroma (Azan stain) showed no signs of inflammation or degeneration. The pulp of the spleens receiving the 5- and 10-mg./Kg. doses of the germicide were slightly fibrotic and the follicles were relatively small. The adrenals showed normal lipoid storage. It was the opinion of the pathologist that all of the changes noted were well within the range of normal variation.

The oral doses tolerated by these dogs without toxic effects would correspond to the daily administration of 300, 600, and 1200 cc. of a 1–1000 solution for a 60-kilogram adult. Considering proper allowances for species differences, we feel justified in concluding that the 1–1000 solution has a wide margin of safety.

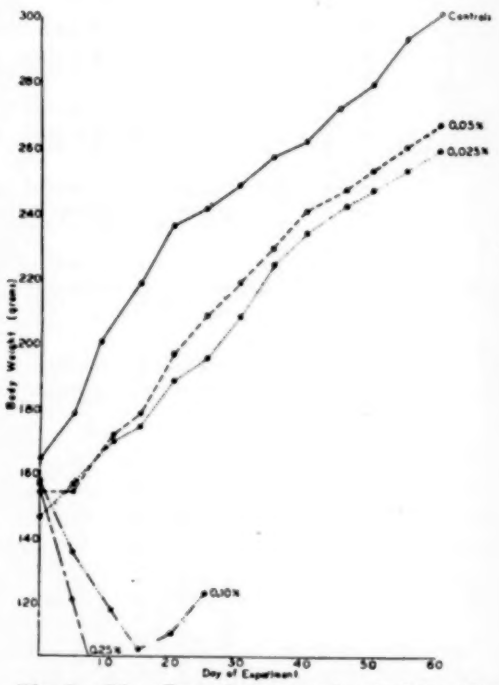


Fig. 2.—The effect of the chronic administration of myristyl γ-picolinium chloride solution as the sole source of drinking water on the body weight of rats.

Skin Irritation in Rabbits.—Small pads of cotton held between layers of gauze, were sewed to the inner shaved surface of a rabbit's ear and saturated with a 3% solution of myristyl-γ-picolinium chloride at 1:00 p. m., 4:00 p. m. and the following day at

8:00 a. m. The pads were left intact and the ears were examined seventy-two hours after the first application of the germicide. The ear was damaged as evidenced by inflammation, edema, and scab formation.

The above experiment was repeated on six ears using a 1% aqueous solution of the germicide. There was only slight scab formation and the edema and inflammation were not marked. A 1% solution of cetyl pyridinium chloride produced the same type and degree of irritation as myristyl-γ-picolinium chloride. From this we conclude that the 1% solution has good skin tolerance, but by the severe conditions of the test the 3% solution produces skin irritation.

Irritation in Rabbit's Eye.—Irritation tests, using a solution of myristyl-γ-picolinium chloride in physiological saline, were made by dropping 0.5 ml. of the germicidal solution into the conjunctival sac of a rabbit's eye and allowing the excess to drain out after thirty seconds. No attempts were made to wash out the excess antiseptic. The conjunctiva were observed for a period of six hours. It was found that dilutions of 1–3000 were only slightly irritating to the conjunctival mucous membrane but concentrations of 1–2000 or greater produced increasing degrees of inflammation.

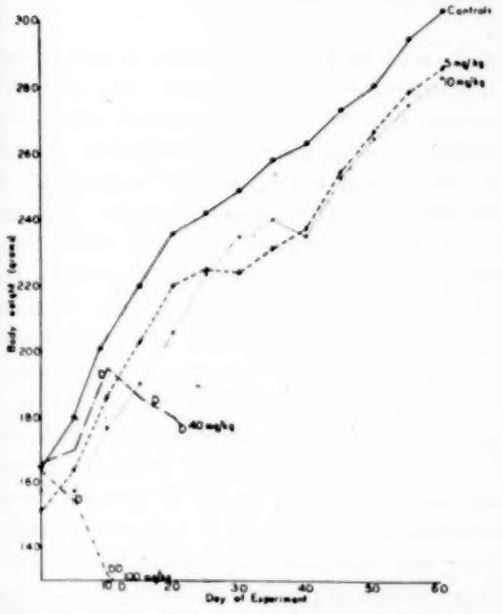


Fig. 3.—The effect of the chronic administration of myristyl γ-picolinium chloride solution by stomach tube on the body weight of rats.

Bladder Irritation in Rabbits.—Myristyl-γ-picolinium chloride was injected through an abdominal incision into the urinary bladder of rabbits weighing approximately 2 Kg. in 0.02, 0.05, 0.10, 0.20, and 0.40% aqueous isotonic solutions in doses of 10 ml. and allowed to remain until voluntarily voided. The rabbits were sacrificed twenty-four hours later and the bladders examined microscopically. With the 0.4% solution, which was the highest concentration tested, histological section of the bladder revealed normal epithelium, submucosa, muscularis, and serosa. There was no evidence of inflammation.

At all concentrations the epithelium and submucosa were normal. In some of the rabbits, which happened to be those receiving the lower concentrations of the germicide, a slight edema of the serosa and muscularis was observed which was obviously due to the method of administration.

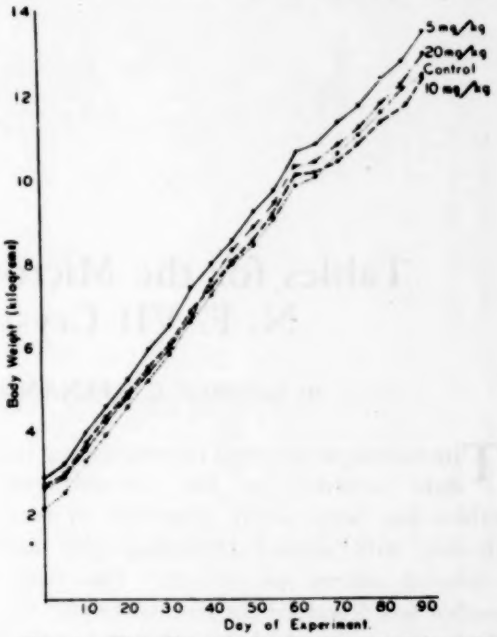


Fig. 4.—The effect of the chronic administration of myristyl γ-picolinium chloride in the diet on the body weight of dogs.

SUMMARY

The LD_{50} of myristyl-γ-picolinium chloride in acute experiments in rats is 200 mg./Kg. subcutaneously, 7.5 mg./Kg. intraperitoneally, 30 mg./Kg. intravenously, and 250 mg./Kg. orally. Corresponding LD_{50}'s for cetyl pyridinium chloride are: 250 mg./Kg. subcutaneously, 6 mg./Kg. intraperitoneally, 30 mg./Kg. intravenously, and 200 mg./Kg. orally.

When myristyl-γ-picolinium chloride was administered mixed with the diet, rats tolerated average daily doses of 17, 30, and 60 mg./Kg. for sixty days with normal growth. When added to the drinking water daily doses of 31 and 52 mg./Kg. produced

no toxic effects or significant inhibition of growth. Single daily doses of 5 and 10 mg./Kg. by stomach tube produced no toxic effects over a period of sixty days.

Dogs twelve weeks of age received myristyl-γ-picolinium chloride mixed with the diet in daily doses of 5, 10, and 20 mg./Kg. over a period of ninety days. Growth was equal to that of the control and histological section of the tissues at autopsy revealed nothing abnormal.

Myristyl-γ-picolinium chloride in 0.1 per cent solution applied as a wet dressing to the shaved rabbit's ear produced only minimal irritation after seventy-two hours. A 1–3000 solution of myristyl-γ-picolinium chloride was well tolerated in the rabbit's eye and solutions up to 0.4% were non-irritating to the mucous membrane of the urinary bladder of rabbits.

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Tables for the Microscopic Identification of N. F. VII Crystalline Substances*

By GEORGE L. KEENAN and WILLIAM V. EISENBERG

THE technique involved in determining the data recorded in the accompanying tables has been amply described in texts dealing with optical crystallography and chemical microscopy (1, 2). The senior author has described the method briefly in a former paper in which he tabulated optical crystallographic data for the crystalline substances listed in the U. S. Pharmacopœia XII (3).

The specialist in this particular technique will observe that in many instances where the optical crystallographic data of synthetic substances are given for the first time, all of the indices of refraction have not been determined. For instance, in the case of a substance that would be expected to have n_α, n_β, and n_γ values, possibly only two will be given, namely n_α and n_γ. Also, frequently a significant index, but not a conventional one, n_i (intermediate refractive index, as between n_α and n_β or n_β and n_γ), will be recorded.

Workers acquainted with this method are well aware that the orientation of the crystal fragments plays an important role in a determination of the conventional refractive indices. Indices measured without regard to orientation would usually be considered worthless. Unfortunately, many organic crystalline substances, especially when they occur in platelike or irregular fragments, do not furnish the worker with the interference figures upon which he must rely to interpret properly the index values he has obtained. There are cases where it is practically impossible to obtain the proper orientation for the measurement of specific indices. Many substances also will persist in assuming one orientation only. Such a feature is significant and valuable for determinative purposes, although possibly disconcerting from a theoretical standpoint.

In this collection of new data, therefore, there will be instances where the n_β was not recorded, because interference figures were not characteristic of the substance, or only partially so, and the suitable orientation for the measurement of this index was not obtainable.

* Received Oct. 24, 1945, from the Food and Drug Administration, Federal Security Agency, Washington, D. C.

TABLE I.—SUMMARY OF REFRACTIVE INDICES AND OTHER DATA BY SUBSTANCE

Substance	System	n_α	n_β or n_i	n_γ	Opt. sign.	Ax. Angle	Dispersion	Orientation
Aluminum sulfate 18 (H_2O)	Mon.	1.474	1.476	1.483	..	.....	...	.....
Ammon. bromide	Isomet.	1.712	...	...	..	.....	...	.....
Ammon. iodide	Isomet.	1.703	...	...	..	.....	...	.....
Ammon. salicylate	?	1.570	1.695	>1.695	..	.....	...	Ext. = par.
Arecoline hydrobromine	?	1.555	1.590	1.655	..	.....	...	Ext. = par. Elong. = Neg.
Brucine sulfate	?	1.512	1.595	1.688	..	.....	...	Ext. = par. Elong. = +
Calcium hypophosphite	Mon.	1.543	...	1.578	..	.....	...	.....
Carbromal[a]	?	1.520	1.533	1.601	..	.....	...	.....
Cinchonidine sulfate	Mon.	1.562	1.604	1.660	..	.....	...	Ext. = par.
Cinchonine sulfate	Mon.	1.587	1.641	1.667	..	.....	...	Ext. = par.
Cinchophen[b]	?	1.545	1.575	>1.734	..	.....	...	.....
Ethylhydrocupreine hydrochloride	?	1.513	...	1.619	..	.....	...	.....
Iodoform	Hexag.	1.750(ϵ)	...	1.800 (ω)	..	.....	...	.....
Lithium benzoate	?	1.585	...	1.640	..	.....	...	.....
Lithium carbonate	Mon.	1.428	1.567	1.572	..	.....	...	.....
Methyl parahydroxybenzoate	Mon.	1.585	1.689	1.700	—	.....	r > v	.....
Morphine hydrochloride	?	1.540	1.590	1.635	..	.....	...	Ext. = par.
Papaverine hydrochloride	?	1.555	1.734	>1.734	..	.....	...	.....
Phenothiazine	Ortho.	1.610	1.734	>1.734	..	.....	...	.....
Pilocarpine hydrochloride	?	1.513	1.570	1.594	..	.....	...	Ext. = par.
Potassium chlorate	Mon.	1.408	1.517	1.523	—	2V = 28° 15′ 2E = 43°	r > v	{Ax. pl. (010) {X∧c = 57½° in obtuse β
Potassium guaiacol sulfonate	Mon.	1.516	1.552	1.627	..	.....	...	.....
Potassium sulfate	Ortho.	1.494	1.495	1.497	..	2V = 67° 20′ 2E = 111°	r > v	..... Z = c Ax. pl. a (100)
Quinine[c]	Ortho. (?)	1.620	1.625	1.630	..	.....	...	Ext. = par.
Quinine phosphate[d]	?	1.575	1.595	1.655	..	.....	...	Ext. = par.
Quinine salicylate	?	1.602	1.620	1.682	..	.....	...	Ext. = par.
Salicin	?	1.505	1.590	1.603	—	.....	...	.....
Santonin[e]	Ortho	1.589	1.592	1.639	—	.....	...	Opt. pl. (100) Z = b
Strontium salicylate	?	1.485	...	1.693	—	.....	...	.....
Strychnine (9)	Ortho	1.61	1.68	1.74	—	2V = large	...	Opt. pl. (100)
Strychnine nitrate (10)	Mon.	1.610	1.624	1.675	..	2V = large	r > v	Elong. = neg.
Strychnine phosphate (10)	Mon.	1.589	1.597	1.655	..	2V = moderate	...	Elong. = ±
Sulfomethane	Mon.	1.518	1.541	1.572	..	.....	...	.....
Sulfonethylmethane	Mon.	1.504	...	1.549	..	.....	...	.....

[a] According to Watanabe (4), it is dimorphic.
[b] Watanabe (5) reports these indices: 1.545; 1.590; 1.630.
[c] The trihydrate which is official, readily loses water of crystallization and the values are those reported by Wherry and Yanovsky (6).
[d] Shaner and Willard (7) report these indices: n = 1.58, n = 1.64.
[e] Mayrhofer (8) reports: slightly soluble in oily immersion liquids and lowest refractive index most useful for determinative purposes.

TABLE II.—DETERMINATIVE TABLE ARRANGED ACCORDING TO ASCENDING VALUE OF THE LOWEST INDEX

n_α	n_β or n_i	n_γ	Substance
1.408	1.517	1.523	Potassium chlorate
1.428	1.567	1.572	Lithium carbonate
1.474	1.476	1.483	Aluminum sulfate
1.485	...	1.693	Strontium salicylate
1.494	1.495	1.497	Potassium sulfate
1.504	...	1.549	Sulfonethylmethane
1.505	1.590	1.603	Salicin
1.512	1.595	1.688	Brucine sulfate
1.513	1.570	1.594	Pilocarpine hydrochloride
1.513	...	1.619	Ethylhydrocupreine hydrochloride
1.516	1.552	1.627	Potassium guaiacolsulfonate
1.518	1.541	1.572	Sulfonmethane
1.520	1.533	1.601	Carbromal
1.540	1.590	1.635	Morphine hydrochloride
1.543	...	1.578	Calcium hypophosphite
1.545	1.575 (n_i)	>1.734	Cinchophen
1.555	1.590	1.655	Arecoline hydrobromide
1.555	1.734 (n_i)	>1.734	Papaverine hydrochloride
1.562	1.604	1.660	Cinchonidine sulfate
1.570	1.695 (n_i)	>1.695	Ammonium salicylate
1.575	1.595	1.655	Quinine phosphate
1.585	...	1.640	Lithium benzoate
1.585	1.689	1.700	Methyl parahydroxybenzoate
1.587	1.641	1.667	Cinchonine sulfate
1.589	1.592	1.639	Santonin
1.589	1.597	1.655	Strychnine phosphate
1.602	1.620 (n_i)	1.682	Quinine salicylate
1.610	1.624	1.675	Strychnine nitrate
1.61	1.68	1.74	Strychnine
1.610	1.734 (n_i)	>1.734	Phenothiazine
1.620	1.625	1.630	Quinine
1.703	...	...	Ammonium iodide
1.712	...	...	Ammonium bromide
1.750 (ϵ)	...	1.800 (ω)	Iodoform

For the determination of the minimum and maximum index values, those fragments were selected which showed the strongest double refraction. In other words, the lowest index of refraction obtainable on the substance would be considered as n_α and the highest as n_γ. Fortunately, substances crystallizing in rods and needles and showing parallel extinction, as will readily be appreciated, do not offer such difficulties.

In the last analysis, the worker interested in identifying a substance by this technique has to take the substance as he finds it, and the quickest approach to the solution of his problem, consistent with accuracy, will be dependence on those data that are readily determined, not on those that are theoretically possible yet at the same time impracticable of measurement and too time-consuming, if they can be determined.

In the accompanying descriptive tables some crystalline substances have been omitted. These substances are variable in composition and are affected by light, or they become hygroscopic or effloresce under certain conditions. In some instances, the substances omitted are essentially microcrystalline in character, precluding any accurate microscopic study. Data on any of these substances would be considered unreliable and at best only approximate.

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